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QUALITY CONTROL IN CLINICAL CHEMISTRY*

By ESTHER F. FREIER, M.S., M.T. (ASCP) and
VERNA L. RAUSCH, M.S., M.T. (ASCP)
University of Minnesota Hospitals, Minneapolis, Minnesota

For more than ten years, this laboratory has been experimenting with a variety of quality control measures to achieve a high degree of reliability of the clinical chemistry results reported to the clinician. Much of the work is performed by medical technology students. That control measures are necessary is emphasized by the accuracy surveys conducted by Belk and Sunderman[3] and more recently by Wooten and King.[29] Even methods as widely performed as the nitrogen and glucose determinations, which have been conducted in clinical laboratories for 35 years, were shown to be very inaccurate in many hospital laboratories.

Although a pooled serum control run with each batch of a particular determination has proved to be one of the best quality control measures, it alone has not been adequate. Using simple statistical techniques and values obtained on a homogeneous pooled control, however, a laboratory can evaluate its performance in terms of confidence limits to be applied to the control pool and to individual unknown specimens and can express the latter in terms of allowable error in per cent. Recently, other laboratories[5, 17, 18, 26] have stressed the usefulness of pooled controls and have published information on their respective allowable errors for several clinical chemistry determinations. It was found that these allowable errors varied from one report to another for a given determination, as did the percentage of batches rejected on the basis of the control value. Since these two parameters, both useful in evaluating the quality of a laboratory, were found to differ in laboratories using control pools, it should be obvious that there is more involved in reliability control than the simple addition of a pooled serum control to each run of a determination.

This paper proposes to review all the measures currently used in this laboratory to insure the reliability of clinical chemistry results, and where possible, to compare such reliability data with the results of others.

Methods and Materials

A. Control Solutions

1. Preparation of Solutions Used for Control

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a. Pooled Control Serum

Clear serum, free of hemolysis, icterus, or lipemia, is collected at the end of each day and frozen until two liters have accumulated. This pool is then thawed, centrifuged, and mixed by gentle swirling in a large container for about ten minutes. Two ml. aliquots are dispensed into vials and corked tightly. No preservative is used. The serum is stored immediately in a freezer at —10° C. and used for all determinations except as noted below.

b. Pooled Protein-Free Filtrate

Protein-free filtrates are collected after glucose analyses have been performed for preparation of the control for blood glucose determinations. Aliquots are packaged in a similar manner to the control serum above.

c. Pooled Control Serum at Different Levels

To avoid contamination, only sera on which protein-bound iodine determinations have been run are pooled as a control of this procedure. These sera are pooled for hypo-, hyper-, and euthyroid levels.

d. Chemical Solution of Known Concentrations

A solution of sodium bicarbonate in a concentration of 25 meq/1 is prepared to control the bicarbonate procedure. This solution is tested daily from a stock bottle kept at room temperature. Although this solution is prepared from the pure dry chemical, it is not considered to be a reference standard since its value is not used to calculate the values of the unknown specimens. The method is independently standardized against standard hydrochloric acid prepared from the constant-boiling acid. With the present bicarbonate method there is no advantage to a chemical control over a serum control.

e. Animal Products

A 6 per cent solution of bovine albumin prepared from Armour's 30 per cent solution* is used to control the total serum protein determination. This solution is prepared in 25 ml. aliquots and preserved by refrigeration.

2. Standardization of Control Value

A new control solution or pool is run in parallel with the control solution currently in use for a period of time to establish its value.

3. Use of Control Solution

An aliquot of the proper control solution is included in each batch of unknown determinations and subjected to the same set of experimental conditions and reagents as the unknown specimens. The value obtained on the control is used to judge the reliability of the values obtained on the unknown specimens.

B. Replicate Analyses

The control solution is analyzed routinely in duplicate for each procedure. In addition, one of the unknown specimens selected at random is run in duplicate. In some procedures all analyses are run in duplicate, as indicated in Table 1.

* Armour Laboratories, Armour and Company, Chicago 11, Illinois.

TABLE 1

Summary of the Number of Reliability Measures Run Per Batch for Representative Chemical Determinations*

PROCEDURE	Pairs of Duplicates	Standards	Recoveries	Control Solutions
Urea Nitrogen (10)	1	3	2	2
Glucose (6)	1	4	..	2
Bicarbonate (26)	1	2	..	2
Chloride (22)	1	2	..	1
Sodium†	all	2/spec	..	1
Potassium‡	1	2/spec	..	2
Creatinine (20)	1	3	occ.	2
Uric acid (14)	1	3	occ.	2
Phosphorus (8)	1	3	occ.	2
Calcium (18)	all	2	occ.	2
Total proteins (21)	1	..	..	2
Albumin (28)	1	2	..	2
Thymol turbidity (21)	..	..	..	2
Bilirubin (19)	..	..	..	2
Cholesterol (1)	all	6	2	2
Protein-bound Iodine (2)	all	4	..	6
Acid phosphatase (15)	all	5	..	2
Alkaline phosphatase (15)	all	5	..	2

* All superscript numerals refer to reference sources of methods pertaining to each procedure.
† Coleman Flame Photometer. ‡ Baird Flame Photometer.

C. Standard Solution

Wherever possible, standard solutions prepared from pure, dried chemicals are run with each batch of analyses, and these are used as reference values in the calculation of the unknown specimens. Using standard solutions to standardize each batch of analyses, rather than relying on a permanently established calibration, compensates for many technical variations in the conditions from one run to another, e.g., temperature, age of reagents, timing, etc.

For the determinations of sodium and potassium the procedure is standardized for each unknown, rather than for each batch of unknowns; each unknown reading is bracketed between two known standard solutions.

D. Recoveries

As a principal example, recovery of added urea is incorporated into each batch of blood urea nitrogen determinations in order to insure optimal activity of the enzyme urease used in the procedure. The level of urea added for the daily control of the procedure is 20 mg. per 100 ml. expressed as urea nitrogen. In addition, to guarantee adequacy of the procedure for pathological values, each new solution of enzyme is tested with enough added urea to correspond to a level of 300 mg. urea nitrogen per 100 ml.

In a similar fashion, recovery of standard solutions added at various steps of the procedures is rotated among the following determinations: creatinine, uric acid, phosphorus, and cholesterol. This is done to demonstrate to medical technology students the usefulness of this method of assaying the accuracy of a procedure. All methods in this laboratory have been tested in this way.

E. Reference Methods

Parallel determinations by two methods are used in selected instances. For example, all paper electrophoreses of serum proteins are checked by the determination of albumin by salt fractionation, and total protein is checked at intervals by micro Kjeldahl determination.[12]

F. "Normal" Values

As each procedure was instituted, normal specimens were run to establish the range of normal values for this laboratory, information that is available upon request. The inclusion of a normal specimen within the batch as a means of control, however, is useless and has, therefore, been abandoned by this laboratory. The physiological range of normal must of necessity have wider limits than the allowable analytical error, for it must include this error. Even the normal control for the prothrombin determination has recently been replaced by a frozen pooled serum control. Biological control of the procedure is retained only for the cephalin-cholesterol flocculation test.

G. Screening of Results for Errors in Calculation and Internal Consistency

All calculations made by one technologist are rechecked by another technologist. Measures have been instituted to detect such individual errors in handling a particular unknown specimen as: the use of a two-ml. instead of a one-ml. pipette, the misreading of a burette or colorimeter, or the actual mixup of specimens within the laboratory. Examples of these internal checks conducted by the technologists are:

a) Comparison of blood urea nitrogen and plasma creatinine values for parallelism;

b) Subtraction of the sum of the bicarbonate and chloride values from the serum sodium value. If differences other than the normal 10 to 13 meq/l are found, the values are rechecked, unless a satisfactory explanation such as uremia, ketosis, or hypoalbuminemia is indicated by other chemical determinations.

H. Statistical Definitions and Formulae Used

Assuming a normal distribution of analytical results on aliquots of the same pooled serum, the standard deviation was adopted as the statistical measure of precision or of the reproducibility of results. Three standard deviation units on either side of the mean should include 99.73 per cent of the determinations.

A *research* laboratory in setting up the methods to be used for a study has time to run relatively large numbers of replicates (usually ten) to measure precision with the traditional formula for the standard deviation:

$$\text{s.d.} = \sqrt{\frac{\Sigma (x - m)^2}{N - 1}}$$

The study then proceeds on the assumption that this figure does not change, a probably correct assumption since the same person usually performs all analyses. But in a *clinical* laboratory, the pressure of the daily work-load does not permit such an idealized assay of precision, nor would this procedure be practical with

student medical technologists who must rotate through the various procedures on a weekly schedule including learning time. Fortunately, statistical techniques have been worked out to permit calculation of the standard deviation from small groups of replicates, and even duplicates. These duplicates may be on random specimens having different values.[24] The average difference between duplicates ($\overline{R}$) is equal to 1.128 times the standard deviation.[30]

To establish the limits for the control solutions in this laboratory, each is run in duplicate for 30 consecutive runs and the results averaged to establish the mean value. The average difference between duplicates ($\overline{R}$) is determined either on the control pool (30 pairs) or independently on 100 pairs of random unknown specimens. The three standard deviation limits for the mean value of the control solution are calculated using the formula: Mean $\pm$ 1.88 $\overline{R}$.[24]

Since the control solution is considered to be representative of the unknown specimens, the statistics derived from variation in duplicates can also be used to calculate confidence limits for the unknown specimens. When unknown specimens are run in duplicate, the confidence limits are defined by $\pm$ 1.88 $\overline{R}$, the same formula used to define limits for duplicate analyses of the control solution. When the unknown specimens are analyzed individually, the confidence limits must be wider to compensate for loss in precision occuring with the omission of the replicate determination. The average of a pair is, in fact, 1.41 times as precise as a single determination, for the gain in precision with replicate analyses is measured by the formula:

$$\text{S.E.m} = \frac{\text{s. d.}}{\sqrt{N}}$$

in which S.E.m is the standard error of the mean, s.d. is the standard deviation, and N is the number of replicates.[30] Therefore, the confidence limits to be applied to an unknown specimen value obtained by a single analysis are described by $\pm$ 2.66 $\overline{R}$. These confidence limits expressed as per cent of the mean of the control pool measure the allowable error of the determination. The allowable differences between duplicates for those procedures in which all determinations were run in duplicate are described[24] by the value 3.27 $\overline{R}$.

Once established, the various control limits are known to all analysts, whether staff or student technologists. The values obtained in the daily run of the control solutions are judged against these limits to determine the acceptability of a set of unknown analyses. If the procedure is considered out of control; i.e., if the variation is greater than would be expected through chance alone, a cause for the difficulty is sought (deteriorated reagent, incorrect wave length, etc.), removed, and all analyses are repeated. Only when no assignable cause is readily found, is a control chart plotted to detect trends that might earlier have initiated the difficulty.

RESULTS AND DISCUSSION

While the use of pooled serum controls in this laboratory dates back to 1948, it is difficult to evaluate the data over the entire period because several methods have been replaced by others shown to be more precise by quality control measures. Thus, methods for determination of spinal fluid protein, acid and alkaline phosphatase, total serum protein, serum albumin, bicarbonate, calcium, and cholesterol have been changed. The data for this paper have been limited to at least one hundred sets collected from February to October, 1957.

The number of determinations added to each batch of the indicated procedures to insure reliability of results is summarized in Table 1. This information should be useful when comparing measures of precision and methods of control from one laboratory to another. The other authors cited[5, 17, 26] have not published similar data.

The data on precision obtained on the control pools is summarized in Table 2. Once established for a procedure, the control limits (column II) remain the same even though new control solutions are introduced. Similarly, the confidence limits for unknown specimens (column III) may be applied effectively over a wide concentration range, provided there has been no major change in technique, such as an additional dilution step.

TABLE 2
Summary and Evaluation of Precision Data Obtained on Control Pools for 18 Chemical Tests

Procedure	I Mean of Control	II Control Limits ±3 s.d.	III Confidence Limits for Unknowns ±3 s.d.	IV Allowable Error in Per Cent Control Mean	V Percentage of Runs "Out of Control"
Urea nitrogen	20 mg/100 ml	± 10%	± 10%	10	7
Glucose	135 mg/100 ml	±5 mg/100 ml	±7 mg/100 ml	5.4	3
Bicarbonate	25.06 meq/l	±0.75 meq/l	±1.06 meq/l	4.2	8
Chloride	108.6 meq/l	±1.6 meq/l	±2.26 meq/l	2.1	3
Sodium	148.5 meq/l	±1.5 meq/l	±2.2 meq/l	1.5	8
Potassium	5.02 meq/l	±0.1 meq/l	±0.14 meq/l	2.8	3
Creatinine	1.78 mg/100 ml	±0.15 mg/100 ml	±0.20 mg/100 ml	11.1	3
Uric acid	5.22 mg/100 ml	±0.16 mg/100 ml	±0.24 mg/100 ml	4.6	25
Phosphorus	4.27 mg/100 ml	±0.17 mg/100 ml	±0.24 mg/100 ml	5.6	7
Calcium	9.62 mg/100 ml	±0.30 mg/100 ml	±0.30 mg/100 ml	3.1	14
Total Protein	6.0 gm/100 ml	±0.2 gm/100 ml	±0.2 gm/100 ml	3.3	20
Albumin	3.02 gm/100 ml	±0.15 gm/100 ml	±0.2 gm/100 ml	6.6	18
Thymol turbidity	12 units	±1.0 units	±1.4 units	11.7	20
Bilirubin	2.3 gm/100 ml	±0.3 mg/100 ml	±0.42 mg/100 ml	18.3	11
Cholesterol	209.7 mg/100 ml	±5.4 mg/100 ml	±5.4 mg/100 ml	2.6	15
Protein-bound iodine (PBI)	5.2 ug/100 ml	±1.0 ug/100 ml	±1.0 ug/100 ml	19.3	10
Acid phosphatase	0.85 KA units	±0.35 units	±0.35 units	41	5
Alkaline phosphatase	17.2 KA units	±1.7 units	±1.7 units	9.9	16

The confidence limits in column III may be expressed as per cent of the mean value of the control (column I) to give the allowable errors in column IV. Unfortunately, the allowable error values have limited usefulness, since the error will change as the mean of the control solution varies, and allowable error unnecessarily tolerates poorer precision at higher levels of concentration. For example, Henry *et al*[11] converted three

standard deviation limits to per cent of the mean and found errors of 11.4, 8.2, and 5.9 for glucose levels of 60, 120, and 430 mg. per 100 ml., respectively, although the variances were homogeneous at the three levels. Allowable error, however, is the most convenient value for comparison with other laboratories.[5,17] Following the practice of the others, the authors have expressed the calculations in column IV to the nearest 0.1 per cent.

The mean of the control pool (column I) falls within the normal range of the procedure in most instances. For serum bilirubin, however, an abnormal level of pigment, obtained by the addition of icteric sera to the pool, is used as the control value because the amount of bilirubin normally present is so small, the readings on the photometer are about 97 per cent transmittance against a blank of 100 per cent. At this low level, one end of the control limits would extend to and be limited at 0 concentration. In addition, the relative analytic error of the photometer is greater at the ends of the measuring scale. Therefore, fluctuations of the control value are more pronounced and more meaningful at a higher level of bilirubin.

Column V lists the percentage of 100 consecutive runs of each determination where the control pool values exceeded the established control limits. These figures represent the percentage of batches that would have been wrongly reported as satisfactory if the reliability measures had not been run. When the control pool value is outside the established limits, the usual practice in this laboratory is to repeat a smaller batch without changing any reagents. It has been our experience that errors in technique associated with inexperienced student technologists or new personnel account for about one-third of the batches that are judged out of control. The repetition of a smaller batch under identical conditions determines whether the poor control is due to errors in technique or to some other cause. If the repeat run is still outside the limits, a systematic search for an assignable cause is made. Because introduction of fresh reagent or standard solutions, or both, is indicated in the daily protocol record, a check of these records frequently shows the source of difficulty. Since all fresh reagents are introduced before the preceding lot is discarded, deterioration or aging causes more difficulties than does faulty preparation of the solution. Difficulty with solutions accounts for about another one-third of the batches out of control. A small number of non-acceptable control values are due to instrument failure. For the remainder of batches out of control no clear explanation is ever found, but may be attributed to fluctuations in temperature and humidity, timing in the various steps of the procedures, and work-load in relation to personnel.

Since reagent difficulties were responsible for a relatively large number of batch failures, an attempt was made to correlate reagent changes with the serial plot of the control values of the protein-bound iodine procedure (Fig. 1). No consistent change in the control values was noted coincident with these reagent changes. During the period shown, however, batches 150, 156, and 160 were out of control. Batch 150 was out of control because of failure of the muffle oven so that ashing was conducted in two stages. The failure of batch 160 was explained by con-

tamination of the freshly distilled water used for the procedure. No explanation was found for the difficulty encountered in batch 156.

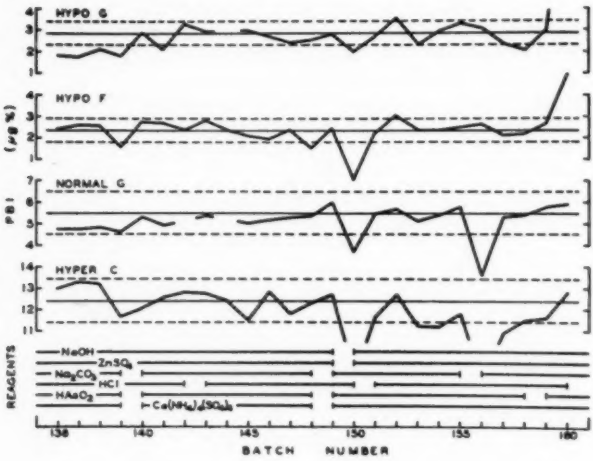

Fig. 1—Control chart for the protein-bound iodine procedure

Occasionally the deterioration of the control serum itself is the cause for a procedure's being judged out of control. Use of pooled serum for quality control assumes stability, but the literature on this point is meager. Connolly[7] reported that the alkaline phosphatase content of frozen serum was stable for seven months. The serum control pool for this determination in this laboratory has been in use for 16 months. Walford et al.[26] studied the stability of frozen plasma for several procedures over a six-month period; they noted no change in the values for total protein, urea, uric acid, creatinine, chloride, or amylase, but they found a 28 per cent decrease in the alkaline phosphatase value. The use of lyophilized serum recently put on the market for this purpose* should eliminate the question of stability of the control pool.

The allowable error in this laboratory for several chemical tests is compared in Table 3 with similar published data.[5, 17, 18, 26] The values from Walford et al.[26] in general are extremely high and possibly include batches out of control because these authors were interested primarily in the stability and overall variation of the control pool values in a six month's period. Since it was their practice to run standard solutions only once or twice a month, their allowable error figures can be used to estimate how much in error the results would be in this laboratory if the reliability control measures listed in Table 1 were not used.

Some of the differences in allowable error from one laboratory to another can be attributed to the choice of methods for a particular determination. Thus, the determination of sodium and potassium by flame

* Labtrol, Versatol, Hyland Control Serum.

photometry is more precise than are the chemical methods used by Benenson et al.[5] In this laboratory the improvement in precision of the chloride titration occasioned by the substitution of a micrometer syringe-burette for the standard micro burette permitted discontinuing duplicate titrations of the unknown specimens without increasing the allowable error. Similarly, the substitution of the cholesterol method of Abell et al.[1] for the Schoenheimer-Sperry[23] technique decreased the allowable error by one-half. From comparison of the figures in Table 3, it appears that replicates for all the unknown specimens are no longer necessary

TABLE 3
Allowable Error—Comparison With Other Laboratories

PROCEDURE	ALLOWABLE ERROR IN PERCENT				
	This Hosp. Lab.	Benenson et al. (5)	Levey and Jennings (18)	Kingsley (17)	Walford* et al. (26)
Urea nitrogen	10	10.9	15	10	23.7
Glucose	5.4	7.1	....	8	27.0
Bicarbonate	4.2	....	9	....	....
Chloride	2.1	3.0	4.0	9.5	7.1
Sodium	1.5	2.9	....	1.4	....
Potassium	2.8	9.0	....	2.0	....
Creatinine	11.0	....	....	10.0	11.1
Uric Acid	4.6	7.0	....	7.0	28.8
Phosphorus	5.6	7.0	....	7.0	....
Calcium	3.3	6.6	....	2.0	....
Total protein	3.3	5.2	4.2	3.0	9.9
Albumin	6.6	7.8	4.2	5.0	16.2
Thymol turbidity	11.7	12.0	....	15.0	....
Bilirubin	18.3	13.3	....	....	46.5
Cholesterol	2.6	8.6	....	10.0	16.8
PBI	19.3	19.2	....	....	....
Acid phosphatase	41	....	....	80	255
Alkaline phosphatase	9.9	14.1	....	35	123

* Coefficient of variation x 3.

with the cholesterol procedure. Another example is given in Fig. 2, which represents parallel determinations on the same pooled serum control for 31 consecutive runs during the same period with both the King-Armstrong[16] and the Kind-King[15] methods for serum alkaline phosphatase. These two methods measure enzyme activity in identical units, but the Kind-King technique yields a smaller allowable error. The comparison in Table 3, however, of allowable error for phosphatase determination is complicated by the fact that the techniques employed by others may be expressed in entirely different units.

Precision, however, should not be the only criterion in the selection of a method. It is possible to have a high level of precision without accuracy, although the reverse is not true. Thus while Kingsley[17] reports excellent precision in the determination of calcium by flame photometry, errors due to interference by sodium, phosphorus, and protein in this analysis make the titration methods more atractive.[6, 9]

The report of Benenson et al.[5] was the only one with which the percentage of rejected runs could be compared (Table 4). These data, which reflect primarily the variation between batches rather than within a batch, serve as a valuable stimulus to improve the methods used in the

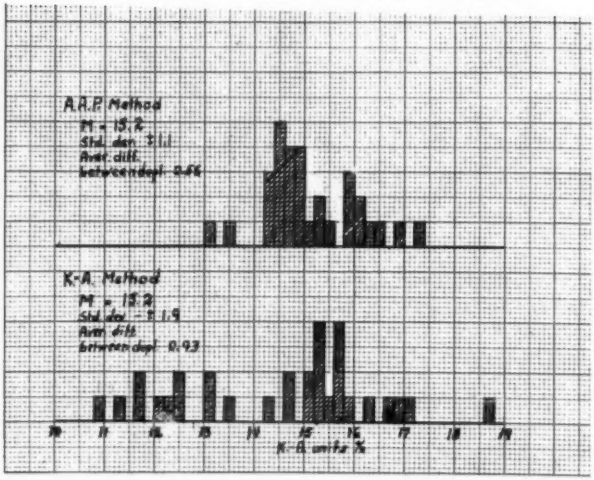

Fig. 2—Comparison of precision on 31 consecutive runs of the amino-antipyrine and King-Armstrong methods for the alkaline phosphatase procedure

laboratory so that the number of runs requiring repetition is decreased. In general, the aim in this laboratory has been to improve technique rather than to widen the control limits and allowable error. One such example is this laboratory's adoption of the gluconate and persulfate stabilizers for the Nessler's reaction in the urea nitrogen determination,[10] which to a large extent decreased the percentage of runs out of control.

The high value in this laboratory for the uric acid procedure (Table 4), is explained in part by a reageant difficulty more elusive than the usual deterioration of solutions. During the period selected for study, difficulty was experienced with several different preparations of the silicate-glycerin solution used to stabilize the uric acid method, all made with sodium silicate solution obtained from Mallinckrodt Chemical Works. The use of sodium silcate marketed by Fisher Scientific Company and J. T. Baker Chemical Company eliminated t'.e difficulty.

It is striking that the other procedures in which the percentage of runs out of control in this laboratory are higher than the comparison values are those in which standard solutions are not included in each batch; the unknown values are obtained from an established calibration curve. An apparent exception to the better control allowed by the use of reference standards in each run is indicated by the figure for the albumin method in Table 4. It should be noted, however, that because the value for the albumin standard was obtained from the established total protein curve, one would expect difficulty to the same extent with both methods. Benenson and his associates[5] have discussed the use of reference standards in preference to established curves. In the total

TABLE 4
Comparison of Percentage of Runs Out of Control in This Hospital Laboratory With Those of Benenson et al. (5) for 15 Chemical Procedures

Procedure	This Lab.	Benenson et al.	Procedure	This Lab.	Benenson et al.
Urea nitrogen	7	17.7	Total protein	20	9.6
Glucose	3	10.3	Albumin	18	10.9
Chloride	3	12.5	Thymol turbidity	20	17.9
Sodium	8	12.3	Bilirubin	11	7.0
Potassium	3	11.0	Cholesterol	15	13.7
Uric Acid	25	8.9	PBI	10	10.9
Phosphorus	7	10.4	Alk. phosphatase	16	12.1
Calcium	14	13.2			

serum protein determination they found an increase from 9.6 to 17.5 in percentage of runs rejected when the established curve was used; similarly there was an increase from 13.7 to 37.0 per cent of runs out of control when the cholesterol determination was calculated from the established curve rather than from reference standards within the batch. To avoid this increased frequency of error commercially precalibrated photometers must be recalibrated routinely using standard solutions.

The matter of individual specimen errors continues to present problems in quality control. A review was made of 270 sets of sodium, bicarbonate, and chloride values that were repeated during one year because of apparent inconsistency of the results. (This number represents about 4.5 per cent of the yearly total of these determinations in this laboratory.) Individual errors were found in 54 sodium, 11 bicarbonate, and 24 chloride values for a total of 89, or 1.5 per cent of the volume. Since the pooled serum control indicated that the batch was satisfactory, these individual errors were the results of mixup of specimens, use of the wrong size pipette, misreading of a burette or photometer, or mistakes in transcription or calculation. Since the three separate items (sodium, bicarbonate, chloride) are determined by three individual analysts and since a check on the validity is made by requiring a mathematical relationship among these three (see G, b, page 5), it follows that any bias of an individual analyst would lead to a greater incidence of inconsistencies than was actually found. The question of bias, however, should always be raised when the control values are known to the analysts. To eliminate this problem, blind or unknown control solutions should be inserted in each run. One objective in using known controls in this laboratory, however, is to teach student medical technologists the means of control and to develop their judgement of quality of work. Students are encouraged to evaluate the quality of their work in terms of the control values and to decide on the acceptability of the batch before consulting with a supervisor. Upon completion of training, graduates of the program have instituted similar control measures in the laboratories in which they are employed, not only to insure the reliability of the laboratory work but also to preserve their self-confidence. The technical difficulty of supplying a sufficient number of blind pools must also be considered. Now that commercial lyophilized sera are available for this purpose, a statistically designed study using this material for blind controls in addition to the known control measures is contemplated.

It is beyond the scope of this paper to discuss in detail work-load factors. The point should be made, however, that the usual practice of counting only the volume of unknown determination gives no credit to the laboratory for the work involved in quality control. The additional work of including the controls listed in Table 1 is small in relation to the total volume of determinations, but that involved in repeating unsatisfactory runs and detecting and eliminating trouble is not insignificant. Therefore, consideration should be given this matter when estimating personnel needs.

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ISOLATION OF MYCOBACTERIUM TUBERCULOSIS USING BLOOD AND PLASMA MEDIA*

STANLEY J. ZACHARIAS, M.S., M.T. (ASCP)
The Meyers Clinic-Broaddus Hospital Laboratories, Philippi, West Virginia

The purpose of this paper is to present two new media for the isolation of the tubercle bacillus and our experience with them.

The media are easily prepared and economical to use. The literature on these media indicates that they are capable of isolating the tubercle bacillus as well as, if not better and sooner than, the more complicated and expensive glycerin-egg-potato media.[5, 6, 7, 8, 11]

At the present time specimens received in the laboratory are digested when necessary and the following media inoculated:

(a) Petragnani
(b) Lowenstein
(c) Penicillin blood agar (PBA) without neutral red.
(d) Penicillin plasma agar (PPA) without red blood cells to which neutral red has been added.

Following inoculation all media are placed in an incubator at 37° C. in a horizontal position for one hour after which they are placed in a vertical position. The reason for doing this is to permit the specimen to be in contact with the entire surface of the slant for a period of time and have the specimen more or less evenly distributed over the media.

The penicillin blood agar (PBA) medium without neutral red is prepared according to the method suggested by Tarshis[7] which is as follows:

Blood agar base (Difco)	3.0 gm.
Glycerol (analytical grade)	1.0 ml.
Outdated blood bank blood (with citric acid solution)	30.0 ml.
Distilled water	69.0 ml.
Penicillin G (potassium)	100 units/ml.

The glycerol and distilled water are mixed, the agar is added, dissolved, and sterilized in an autoclave at 15 pounds pressure for 15 minutes. The medium is cooled to 45° C., at which temperature the blood bank blood and penicillin are added aseptically, mixed thoroughly, and adjusted to pH 6.8. This mixture is then distributed into sterile screw-cap culture tubes, slanted and allowed to solidify. The tubes are sealed with masking tape, dipped into hot paraffin (to conserve moisture), placed in an incubator for three days to determine the sterility of the media, and stored in a refrigerator.

We used whole blood which was aseptically obtained by venipuncture and found that it could be substituted equally well for citrated outdated blood bank blood. We did this to determine whether the anticoagulants were necessary for the isolation of the tubercle bacillus because all laboratories do not have blood banks and therefore outdated blood is not available for this purpose. Blood from a bank which is older than eight weeks should not be used.[10]

A search of the literature was undertaken to determine whether the concentration of blood in the penicillin blood agar (PBA) medium was critical for the isolation of the tubercle bacillus, and it was found that various investigators used concentration from 15-50 per cent.[1, 6, 7, 11,

* Reprinted from West Virginia Microscoop.

It also was found that the plasma concentration in the penicillin plasma agar (PPA) medium could be varied,[1,9] and that some investigators have added malachite green as well as penicillin (to inhibit contaminating organisms).[1, 11] In this laboratory, we have not used malachite green in our blood and plasma media, and therefore can not report about it, except to say that it has been found to be able to inhibit the tubercle bacillus in certain concentrations.[1] Tarshis suggests using 0.01 per cent malachite green or 0.005 per cent sodium tellurite in the media if contamination becomes a problem.[10]

On the basis of the cytochemical reaction of neutral red with the Mycobacteria,[2, 3] Sparkes[9] has substituted plasma for blood and added neutral red to the medium. The purpose of adding the dye is that virulent organisms absorb and bind the dye, producing pink colonies, while the avirulent organisms do not absorb the dye, and, as a result, the colonies are white or yellow.

The medium recommended by Tarshis[12] which has been modified by Sparkes and to which neutral red has been added is prepared as follows:

Agar	1.5 gm.
Neutral red (0.05 per cent) aqueous	6.0 ml.
Glycerol	1.0 ml.
Plasma (from blood bank)	25.0 ml.
Distilled water	74.0 ml.
Penicillin	50 units/ml.

This medium is mixed in the same manner as the blood agar medium given previously in this paper except that blood plasma is substituted for whole blood and neutral red is added.

At the present time we are using the four media for the isolation of the tubercle bacillus. In the preparation of the blood and plasma media we use either formula as given, except we substitute whole blood or plasma (with neutral red) as desired. It was found that either media could be modified and support the growth of Mycobacteria.

Our experience with the various media indicates that no one media was superior to any of the others. In no instance were we able to isolate the tubercle bacillus on all four media from a single specimen, and on one occasion we were able to isolate the tubercle bacillus on the blood and plasma media, while the organism was not isolated on Petragnani or Lowenstein media.

The blood and plasma media, in our opinion, support good growth of the tubercle bacillus as compared with the Petragnani and Lowenstein media, are economical to use, and easy to prepare. In addition, the plasma medium with neutral red can be used as an aid in the identification of virulent from avirulent forms of Mycobacteria.

It was the experience of Haley et al[4, 5] and Leibovitz et al[6] to find that Tarshis blood agar yields positive cultures earlier than those obtained with modified Lowenstein-Jensen medium.

Lango and Fleischmann[7] recommended the combination of American Trudeau Society medium and penicillin blood agar (PBA) medium for the routine detection of the tubercle bacillus. They further recommended penicillin blood agar (PBA) medium if only one is to be used. The reason for this is the ease of preparation, economy, and the high yield of positive cultures.

SUMMARY

The use and preparation of two new media for the isolation of the tubercle bacillus have been given, and a modified medium for the differentiation of virulent from avirulent Mycobacteria has been presented. These media are economical and easy to prepare.

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YEAR OF THE TURNING POINT: 1958*

LEONARD A. SCHEELE, M.D.

Chairman, Commission on Health Careers, National Health Council; President, Warner-Chilcott Laboratories, Morris Plains, New Jersey; and Former Surgeon General, U. S. Public Health Service

The opportunity to join with you in this opening session of your 1958 meeting is both a pleasure and an honor—a pleasure to meet so many friends, new and old; an honor to be called upon to try setting my hand at a keynote for this important occasion.

But that is not all. In inviting me here, you have given me a challenging—and stimulating—assignment. Challenging, because I would like to discuss with you the points at which our paths—our professional interests—come together; and that takes in practically all the great issues in the health field today. Stimulating, because these meeting points represent a converging of interests—a recognition of the common problems and common goals which are shaping the nation's health services and health professions.

As a long-time friend of your profession, I shall have something to say this morning about medical technology and its increasing professional importance.

As Chairman of the Commission on Health Careers, I shall bring you a report on the National Health Council's Health Careers Program and some perspectives on the task ahead in Health Careers.

As President of Warner-Chilcott Laboratories, I shall take this occasion to make a special announcement—the establishment by my firm of a new kind of scholarship in medical technology.

The first of its kind, this scholarship is, I believe, a development of real importance to the American Society of Medical Technologists, as well as to Warner-Chilcott. It is a step toward recognizing one of your most critical needs in professional education. It symbolizes a way for new advances toward broadening the career potentials in your field. It represents in its way recognition that American business is interested in careers in the health profession.

But the importance of this scholarship is not limited to your profession or to Warner-Chilcott. It has a broader significance—because it demonstrates, and contributes to, this convergence of interests which is really the thread, the connecting link, in everything I want to discuss with you today. This sharing of common interests and problems is bringing people closer together throughout the health field. As these lines of interest converge, we may well see a major turning point for medical technology and for the health professions generally.

Even now, when the year has still not quite reached the half-way mark, we are already seeing some new and encouraging directions. By the time 1958 comes to a close, perhaps we will be justified in calling it the year-of-the-turning point, in spite of the problems that still lie ahead.

Medical Technology Moves Ahead

As a "young" but mature profession, medical technology has made significant strides. Both its progress and its problems represent trends

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and needs, opportunities and obstacles, that are part of an evolutionary process—the growing pains and the coming of age experienced by every health profession.

We in the health professions, as well as others, would no doubt learn a lot about themselves if we "could see ourselves as others see us." You would not need to do any viewing-with alarm if I could hold such a mirror before this gathering. As a matter of fact, *my* view from this platform suggests that "seeing yourselves as *I* now see you" would be a very rewarding experience for members of your profession.

Though I have no magic mirror, I would like to tell you something which may serve the same purpose—and may do an even better job, because it lets us see ourselves through the eyes of one of the nation's great scientists—Dr. Vannevar Bush. In a recent talk before the American College of Surgeons, Dr. Bush set himself the task of answering a question of critical importance to all of us in the health field, as well as to his own colleagues in the physical sciences.

The question he posed was: "What *is* a profession?"

Here are the main characteristics—the duties and responsibilities and prerogatives—which, in his view, are the distinctive marks of every true profession. Perhaps you, who represent the profession of medical technology, would like, so to speak, to "try them on for size":

First, Dr. Bush says, the members of any true profession are the possessors and custodians of a special field of knowledge acquired by assiduous study. They have an obligation to put this professional knowledge and understanding to good use, to make a lifetime practice of increasing their own skill in its application, and to do their share in expanding the sum total of what is known.

For his next point, Dr. Bush speaks with the authority of one who has had broad experience in national affairs. No one knows better than he that a true profession can never retreat into an ivory tower. As a realist, he does not underestimate the importance of professional status or the symbols and titles by which members of a profession are publicly identified. They are entitled to respect, but this alone is not enough. In his view, the fact that "true members of a profession must detach themselves from the mad scramble for this world's goods," makes it all the more essential that each have the financial wherewithal needed "to live in a reasonable and proper manner."

Dr. Bush is equally explicit in what he has to say about interprofessional relationships—and here he takes the health field as his example. Pointing out that this field embraces a number of interrelated disciplines, he states "that the respect accorded by the public must be shared throughout these groups." He leaves no doubt that he regards this kind of mutual respect as essential in the morale, pride, and loyalty on which good working relationships are dependent.

Having made his position clear on all these points, Dr. Bush comes finally to what he considers "the inviolable hallmark without which no one, regardless of other qualifications, is truly entitled to the proud name of a profession." In his words, *"This primary characteristic of a profession is to minister to people—to serve them with dignity, devotion, and skill."*

If we accept this definition of a profession, I think there is no question that the place of medical technology is alongside the other sister professions in the health field. One of the signs of maturity, for a profession as for an individual, is knowledge that progress sometimes comes slowly.

It might be worthwhile to compare your professional progress so far against the characteristics that Dr. Bush considers essential. You yourselves are the ones to apply his yardstick. But I can take note of the many evidences of progress which a nearby friend like myself can discern.

I know, for example, how constantly the medical technologists gathered here this week—and indeed, the full membership of your Society—have demonstrated what Dr. Bush means when he says that the highest duty of a profession is to minister to people. I pay tribute to your sense of dedication and your often demonstrated strong personal feeling of responsibility for each patient's welfare.

You, yourselves, have moved farther ahead than your colleagues sometimes realize. Just as a family sometimes finds it hard to accept the fact that its younger members have reached maturity, so also, among professional groups there is often a lag in status, in recognition. Even so, you don't have to look very far to see how many encouraging developments there have been just within the past twelve months—among them:

The move towards significant advance in recognition under Federal Civil Service—confirming your professional status is an intrinsic gain, to say nothing of the fact that it provides an important base for further recognition from many other hospitals and hospital administrators. This, as well as pay scale, is a result of upgrading Medical Technology to a professional classification. From my long-term experience in the U. S. Public Health Service, I can fully appreciate what this change means. Anyone who has spent many years in government as I have will always regard the career civil servant with respect. As you know, the Public Health Service includes many people in the health professions under Civil Service.

So too, in education for medical technology, this year has seen some important steps forward—among them, joint consideration, by your Society and the American Medical Association, of increasing educational requirements for entering your field to three years of college before the final year of hospital study. I understand that at least one institution, the University of Minnesota, has gone still further and is formulating a curriculum, as well as standards and requirements, that look toward a Master of Science degree in your specialized field. Although this has not been activated, it holds promise for the future.

In addition to these most recent gains, you have continued progress along these lines which you yourselves may by now almost take for granted. For example, medical technology is one of the health disciplines which has maintained a sound balance between broad basic knowledge and skills and the need for specialization. Both blood bank technology and cytotechnology represent the kind of specializing without splintering that promotes effective utilization of health personnel.

Turning to one other example, I believe you are taking a particularly progressive leadership position among the health professions in opening your doors to the physically handicapped, as well as to young people. Without relaxing standards of competence, you have taken down the barriers which too often exclude competent persons who have physical handicaps. And you are a good deal more realistic than some other groups in adjusting to the womanpower revolution. More flexible age requirements, more willingness to develop part-time working arrangements for married women—in ways like these you are doing your own exploring.

To you who have done the pioneering that makes 1958 a turning point, this should be a year of genuine satisfaction. Since the evidence shows that you are pioneers by nature as well as by necessity, I am sure you will keep up the work, attacking the problems ahead with the same dedication you have put into your task so far. Without trying to itemize everything that may still need to be done, perhaps I might speak from my own experience and suggest a few issues that seem to call for your early and continuing effort. For example:

I commend your purpose in broadening your own career horizons still further—both for the sake of your own personal satisfaction in your profession, and also as a necessity if you are to attract and hold more people of your own caliber—young people and others too.

I would like to suggest that you can make an even stronger case for your profession along these lines: One of the distinctive obligations of all health professions is to apply scientific knowledge to the well-being of individuals. But of all the health disciplines, none perhaps must carry out this duty more directly than the medical technologist. Often the medical technologist is the most visible connecting link between the patient's bedside and the laboratory behind the scenes. This linkage—scientific skill plus personal concern for the patient—makes a strong appeal to the very people whom you most want to bring into your own ranks. Add to this the fact that so much of your work is in exciting areas, like cancer and the virus diseases, where there is still great hazard and great need for scientific exploration. This too, is a point which makes all the difference between "just a job" and a real career.

These same considerations add urgency to the need for broadening the horizons of professional education in your field. I share your concern over the need to provide for additional technical training, and for "teaching the teachers." The question of post-graduate training, in educational method as well as in content, certainly warrants top priority. It affects every one of your 650-odd Approved Schools and every one of their 3000 students.

But strengthening professional education will have more than its share of hindrances and frustrations unless its implications are understood outside of your profession, as well as among yourselves. In recent years, science has moved so rapidly that job names and job classifications within the laboratory have not always kept pace. You all know, I am sure, that the U. S. Census Bureau still uses just one old-time catch-all category—"technicians, medical and dental." Whatever that means, it's all the credit each of you gets as "a statistic."

Even among physicians, hospital administrators, government officials and community health leaders, there sometimes seems to be a blind spot to levels of responsibility in medical technology and the way jobs should be classified.

No doubt many of you have seen the chart comparing medical technology and nursing classifications in the April 1 issue of HOSPITALS. This was prepared by your National Committee on Careers in Medical Technology and, I've been told, will be shown to you later this week at the panel program on recruitment to be given by that group. It was based on a recent Labor Department study of regional differences in hospital salaries. Nursing was listed with separate categories for directors, supervisors, instructors, head nurses and general duty nurses. No gradations for medical technologists were listed. Further, the average salary for regular medical technologists was about at the level of the general duty nurse in most of the 16 cities surveyed, and in six it was even lower. In two places the average for medical technologists make a slightly better showing than that for head nurses.

This doesn't seem to me very realistic—in terms of classification *or* compensation. To quote Dr. Henry Wollenweber's statement in a report on medical technology to the 1957 annual meeting of the pathologists: "It is the complaint of many medical technologists that there is not enough room for advancement in this career. Most laboratories have, in effect, a chief medical technologist. Some have department supervisors, teaching supervisors and instructors. Should an effort be made to develop these positions into strong categories with compensatory income?"

To that question, the obvious answer would seem to be *yes*. Surely it is time to identify these positions and provide more adequate recognition for the training, experience, and responsibility required at each level.

I should make it clear here that I do not mean to imply that nurses are overpaid. Rather I meant to emphasize that technologists are underpaid.

I have cited some of the problems in medical technology which seem particularly urgent to a friend and well-wisher observing your profession as I do. But none of these issues—of manpower shortages and recruitment, of personnel utilization, of status, salary and job satisfaction, of aptitudes, skills and professional education—not one of these problems is yours alone. They are common to most of the health professions. As a matter of fact, these areas of concern that I have just listed represent some of the critical national problems that have recently brought the Commission on Health Careers into being.

Health Careers—The Overall Program

The Commission itself is new. But the National Health Council's Health Careers Program, of which it is a part, is already well-established—your own Society is a member of the Council, and also a charter member in Health Careers. You are one of the 57 professional societies which serve as signatory agencies in the Health Careers Guidebook, and since the start of the initial Health Careers Project three years ago, your members have been active participants in this

nationwide effort to interest young people in the career opportunities health offers.

Without repeating what you already know about Project purposes and activities, I want to bring you up to date—to highlight some of the main points in our current outlook—among them, the relationship between Health Careers Project and the Commission:

Both are parts of a single overall Program.

The Project came first because its job couldn't wait—was already long overdue. As you know, its purpose is to reach the nation's young people, give them the information and incentive they need in order to take account of career opportunities in the health field as they plan their own futures.

But important as it is to open up health career horizons for young people, this is only one approach to the health manpower problem. If we are ever to meet the needs that we have just been discussing—if we are to deal with them across the board, wherever they crop up throughout the health field, we must launch a broad attack on all fronts.

This is why the Commission on Health Careers was established. Its purpose is to consolidate the attack, to provide a rallying ground and take-off base for a full-scale cooperative effort.

One more point to round out this overall background: Just now, in referring to cooperative effort, I was not slipping into a vague generalization. The Health Careers Program was originated by the national health organizations. It is their program. This is true not only of the initial planning and of continuing day-to-day activities, but of financing. The Health Careers Guidebook, together with supporting materials—pamphlets, films and so on—have been produced and distributed with the public interest support of The Equitable Life Assurance Society. The costs of administering the Project are met by contributions from participating national agencies. For the broader program of the Commission, we are, however, looking to additional sources of funds, including contributions from industry and foundation grants. One of the significant things about this Program is the extent to which it represents voluntary initiative and depends upon broad support from all segments of our American society.

Health Careers Project—Progress and Prospects

Within this overall framework, the Health Careers Project is not merely continuing. It is going full steam ahead. Because your organization is a participant in these nationwide activities, I take special satisfaction in giving you a few highlights on current progress and on prospects for the months ahead:

1. *Use of Health Career Materials.* More than 100,000 copies of the Guidebook have been sent out—two out of three of them on individual request—and all of the supporting materials are getting comparable usage. Next steps in utilizing these Health Career materials include a recheck with all high schools to take care of new schools and increased enrollments, as well as the demand for replacements for publications and visual aids worn out by hard usage.

2. *National Agency Participation.* Along with professional societies and both voluntary and governmental agencies in the health field, a number of

other organizations have adopted Health Careers as a national project—among these, one of the most active and helpful is the Woman's Auxiliary of the American Medical Association.

3. *State and Community Health Career Committees.* In addition to schools, agencies and individuals—some 16,000 in all—who have undertaken Health Career activities of one kind or another, more than 200 state and community groups have initiated and are carrying on organized Health Career programs.

Following the pattern of the national Project, many of these bring together substantial groups of state and local agencies, and these participants include many national agency affiliates. As a matter of fact, this "grass-roots" cooperation on the part of national organizations is considerably more extensive than I would have guessed. In a recent sampling, for instance, 89 state and local Health Career programs report participation by 1278 affiliates of 91 national organizations.

Bringing this composite picture closer home, you will be interested to know that your field is represented in 39 of these 89 Health Career Committees, and that together they report participation by 70 of your state and local groups—33 affiliates of the American Society of Medical Technologists, 21 interested in medical technology identified with the American Society of Clinical Pathologists, and 16 representing the Committee on Careers in Medical Technology. And bear in mind that these figures are not complete—because the survey itself was only a sample.

4. *Local Health Careers Information.* Since I have no doubt that most of you have had a hand in the school and community activities in which these Health Career Committees engage, I won't attempt to enumerate or describe them—except to point out one kind of activity that seems to me particularly useful. This is the localizing of Health Careers information. In many places, facts about nearby training opportunities, local scholarships, and so on, reach young people through many channels—best of all, of course, in person-to-person contact. But as an attention-getter and as a take-home piece to share with parents, the printed word plays an important part too. In addition to the usual local publicity, often in the school's own newspaper, as well as the regular press, there has been another development which has proved particularly useful—a growing crop of full-fledged local Health Career publications. About 30 of them have been issued as a direct result of the Health Careers Project—many of them reproducing text and illustrations from the Guidebook. More important still, they follow the Project pattern of covering—if not all the 156 occupations described in the Guidebook—at least a substantial cross-section. It goes almost without saying that most of them include medical technology as a field of major opportunity.

5. *Next Steps.* Objectives identified for special emphasis in coming months look toward a variety of activities—among them:

—Workshop conferences with professional associations and national health agencies on the improvement of recruitment materials and methods;

—Additional opportunities for the exchange of local-action experience, with special attention to ways and means of interpreting Health Careers to parents and enlisting their interest. (A new series of

Health Career bulletins, and hopefully, some regional meetings would be involved in this development).

—Supplementary services to the schools—in line with suggestions made by teachers—to provide Health Career materials for classroom as well as counseling use. (Though these services would take account of ideas and experience coming from many educators—in social studies, health education and other subjects—a major target would be the utilization of Health Career information and activities in high school science teaching).

—On all fronts, continuation of efforts to promote recognition of the health field as a major area of career opportunity for science-minded young people. (This is an increasing, but not a new, emphasis. Last year, for example, through cooperation with the National Science Foundation and the Federation of American Societies for Experimental Biology, Health Career materials, together with a special factsheet for science teachers, reached all of the Summer Institutes for Science Teachers in the health-related sciences. Similar services are being provided again this year. Working with other agencies that have special interest in the health sciences, the Project also hopes to bring this aspect of Health Careers to the direct attention of young people, their parents, and the public generally).

This top priority on interpreting the role of science in the health professions is, of course, a matter of special concern to you. But I needn't tell you that this isn't the only way in which the Health Careers Project underpins your own recruitment efforts. By offering teen-agers an early introduction to the health field and giving them incentive to explore on their own, it increases the number of promising career candidates for all health professions. As evidence that this is what really happens, let me cite just one recent experience:

A syndicated Sunday newspaper feature story describing one health occupation appeared a few weeks ago. It included one brief reference to the Project and gave the mailing address. The surprising fact is not that more than 1000 inquiries were received in the first five days—but that most of them asked for more information about health careers generally, rather than about the particular career featured in the story. Young people do want to do their own exploring, make up their own minds. And with the idealism—the desire to be of service—which is strong in many of them, health makes a big appeal.

Our task is to give them the facts. The Health Careers Project enables the health field as a whole to respond to the demand, by educators and counselors, for information that is comprehensive, as well as authoritative and consistent with sound guidance principles.

As Miss Rose Matthaei, your executive secretary, pointed out in a recent and timely letter, your society has always been enthusiastic about this joint approach. I am sure that she—and all of you—would agree with one of your colleagues who adds that "working together through the Health Careers Program is increasingly helpful now that the number of competing recruitment programs has made school people understandably more resistant to the demands of individual professions." In the words of another national recruitment director, "It is abundantly clear that Health Careers has had a terrific impact on the secondary schools of the

country, and this, in turn, has created interest among young people who had never heard of us before."

Taking account of this terrific impact, Dr. Howard Rusk has called the Health Careers Project "the biggest advance in meeting our health personnel program."

It is a big advance, not only because it provides the means of reaching more and more young people, but also because it has given us a head-start in the broader attack for which the Commission on Health Careers has responsibility. In my capacity as Commission Chairman, here are some of the elements in this experience that seems to me most significant:

—The Health Careers Project has tapped a nationwide reservoir of interest and initiative;

—it has underscored the fact that such an attack involves a lot more than recruitment, essential as that is;

—it has highlighted the gaps in what we know about health manpower needs and what we are doing to meet manpower competition;

—it has demonstrated that the lack of interest on the part of young people has many causes—involving facilities, teachers, earning opportunities, and other problems—and that too little is known about many of these questions;

—it has opened the door to new and effective allies—individuals and groups in other fields who are prepared to stand with the health profession in meeting competition for manpower;

—it has shown that national, state and local health agencies, both governmental and voluntary, can and are working together to attack this common problem;

—and, above all, it has demonstrated that effective action must come from non-governmental leadership if there is to be substantial progress in meeting the health manpower challenge.

The Commission on Health Careers

These are just some of the elements on which the Commission on Health Careers can build. But we do not expect to solve all the problems of health manpower single-handed, or to bring about any overnight miracles. The Commission alone cannot do the whole job, and it has no intention of trying. The work of all agencies with both primary and secondary interest in health problems is required for ultimate success. The Commission has its own work cut out for it, and this in itself represents an opportunity, and an obligation, of national magnitude.

As evidenced by the widely ranging experience of those who have already been named as Commission members, our basic responsibility is to represent the public interest in assuring adequate manpower for the nation's health services.

You may also be interested to know that we plan to bring in additional members. Within the limits of a comfortable working group—say twenty or at most twenty-five—we will continue to draw upon health, education, the physical and social sciences, business, labor, communications, government, family, and civic interests, and other allied areas. The broader our composite experience and background, the better prepared we shall be for our task.

This task charges us with responsibility to examine the health man-

power problem whole, in all its ramifications; to encourage new ways of helping to meet health manpower needs; to stimulate public and professional participation in these efforts; and—most important of all—to serve as a focal point for a concerted and comprehensive attack on this major bottleneck to further health progress.

Before they held their first meeting this spring, the Commission members had already done a good deal of thinking about what is involved in this task and how to take hold of it—thinking marked by down-to-earth, but dedicated, acceptance of the job to be done, as well as willingness and capacity to give imaginative leadership.

They see the health manpower problem in perspective, without bias in favor of any one of the health professions. This impartial and objective view does not minimize the fact that this is a big problem, but it does reinforce the Commission's determination not to get bogged down by sheer "bigness."

As one way of seeing the problem whole—of bringing order and practical common sense to bear on it, we agreed that substantially all the questions that need looking into can be lined up more or less as follows:

1. *Supply of health personnel*—What we know and what we need to know about present and anticipated supply and shortages; about the possibilities and problems of tapping additional personnel sources; about the considerations involved in estimating future needs—and all the related questions that need further exploration.

2. *Utilization*—Without going into the strictly professional problems of personnel utilization, the Commission will want to take a look at some of its broader aspects. To cite just one example: Are the health services making the most of the skilled people they already have? Do they always give professional people a chance to work at their highest level of training and competence? And is it not increasingly necessary to seek these existing professional resources now, in view of the fact that we are heading into a decade when the national supply of skilled younger workers, those in the 20-30 year age group, is actually shrinking?

3. *Job Satisfaction*—Here we suggest bringing together two areas that are often considered separately—what the individual brings to the job—by way of aptitudes, motivation, training, and personal goals; and also what the job brings to the individual—in terms of personal satisfaction, earnings, fringe benefits, opportunities for advancement, etc. All of these questions certainly have a direct bearing on recruitment—and there are few areas where answers are so likely to be based on off-the-cuff assumptions rather than solid facts. We must ascertain the facts.

4. *Education* and *training* for occupations in the health field—this involves not only professional education, but the whole range of problems raised by the explosive increase in school age population, and the crisis this has brought about in primary and secondary, as well as higher education. The strengthening of professional education, is of course, a prime essential. But beyond this, don't we all have an obligation to speak out for "First things first" in all kinds of schools and for all kinds of young people—for better teaching—for better counseling, and for better basic education? No one realizes more fully than those of us in the health field that the nation must look to its schools to turn out well-trained and ma-

ture people competent to serve not just in the top echelon, but all along the line.

5. *Recruitment*—which brings us full circle back to our starting point because success in recruitment depends in large part on how effectively our health professions and health services operate and deal with these other questions—utilization, job satisfaction, education and training. But successful recruitment depends too on the effectiveness of its own communications techniques, its methods and materials. We need to know a lot more than we do here. We need to evaluate present methods and develop new ones. Most of all, perhaps, recruitment depends on how realistic and how statesmanlike the health field is in coming to grips with the basic issues of manpower competition in a time when the entire nation is caught between an unprecedented low in its supply of able young adults and an unprecedented demand for competence, for skills, and for creative imagination.

With this broad preview as a starter, the Commission has laid out a course of action. As it took shape in our discussion, it gives ample scope for meeting the responsibilities with which we are charged. But it is also insistent in its emphasis on manageable activities geared to get practical results. With this as the determining consideration, we see three areas in which we believe Commission action will be particularly useful.

These are: first, *fact-finding;* second, *study and evaluation;* third, *public information.* Within this framework, here are some of the main elements in what we see ahead:

To start with, the Commission will seek facts—the kind of facts that provide ammunition for action. No one, for example, has a comprehensive picture of overall health manpower supply and demand.

In spite of all that you know about personnel needs in medical technology and the similar information available in other fields, we need more data—and clearer definitions—in order to arrive at an overall total to present to the public. Working in collaboration with organizations that have pertinent information, the Commission plans to assemble and publish the facts for the entire health field. In this initial survey, we shall again turn to the professional societies—yours and others—for whatever supply-and-demand information each can provide. To be meaningful, such a compilation should cover regional as well as national data and include estimates of future as well as present needs. This is a large order.

While preparations for this survey are in process, we have, however, already taken a first step with respect to current information on enrollment trends in the professional schools serving their various fields. We all know that these trends represent a significant gauge of what is happening with respect to specialized health personnel.

I am sure, for example, that an important consideration in your own planning is the fact that—even with the big increase in enrollments in approved schools of medical technology—your schools have room, and you have need, for more students. Is your rate of increase—22%, I believe, in the two years from 1954 to 1956—holding up? Is the capacity of your schools still increasing? How do trends in your field compare with those for other health disciplines? In the aggregate, is the 1958 picture encouraging? Relatively simple as this first step is, within the next few weeks it should begin to provide some very useful indicators and guideposts.

In its second area of action—study and evaluation—the Commission will again put its main reliance on professional societies and other competent organizations. Its purpose here will be to stimulate a fresh and imaginative approach to health manpower. With this objective, the Commission will seek, examine, and propose new ways of evaluating health personnel needs and of tackling problems of supply, utilization, job satisfaction, education and recruitment. It will foster experimentation. It will promote the adoption of new, but tested, methods to meet health manpower needs more effectively.

Again, this means working with professional societies, with national agencies, both governmental and voluntary, with departments of education, with universities, professional schools, and other research centers, as well as with appropriate state and local institutions and organizations —including, very possibly, some of the state and local Health Career Committees. As you know, many studies under many different auspices have already been made or are in process. All of us know about some of these. But from the inquiries already made to the Commission, I think it is safe to say that nobody knows about enough of them.

Whatever information along these lines is compiled will, of course, be made available to all interested agencies. We are as concerned with avoidance of duplication as we are with filling gaps. And once gaps are identified, we shall again turn to the organizations and institutions in health and elsewhere who are equipped to undertake whatever new studies or actions are indicated—in the social sciences, in education, in manpower problems, in finance, and in other fields, to develop additional studies.

Since the Commission represents the public interest in health manpower, we feel that public information is one of our primary obligations —an area where we can undertake an action program under our own auspices, as well as through cooperation with professional groups.

The Commission is convinced that a climate of better public understanding is essential if the nation's health manpower needs are to be met. Our public information objective is, therefore, to provide facts and interpretation, to stimulate personal interest and motivation. This is one of the many areas in which there is still a gap between specialized knowledge and public understanding. These gaps—and the public apathy they induce—may very well prove to be one of the most disturbing aspects of our times. In dealing with health manpower, we hope and believe the gap can be bridged. Helping to build this bridge of public understanding is one of our major goals. There never was a time when mutual understanding was more essential—and this applies to *all* of us within the health field, as well as to the people whom we serve. The one most inescapable fact about health work today is that it is caught up on the mainstream of the great changes through which we are living.

The Commission on Health Careers takes full cognizance of this fact. Among the causes of the pressures on health manpower, it cites, for example:

—World events—like the launching of the "sputnik" with its unprecedented emphasis on science careers—highlighting the need to focus this career emphasis on *all* sciences, including those in the health field.

—National developments—the flood of young people into overcrowded and understaffed high schools; the real shortage of people in the 20-to-30 year age bracket; and what such pressures are doing to increase the difficulties of professional education and to step up recruitment competition.

—Increasing demand for health services—because of the need to apply ever-increasing scientific knowledge in disease prevention and treatment and to keep pace with population forecasts of still larger groups of those who require most care—the very young and the very old.

Looking Ahead

These are the terms in which the Commission sees its job. As you see, they are very specific and very practical—realistic next steps in meeting a problem of far-reaching significance.

I like to think—and I like to believe I am justified in thinking—that the same general terms apply to the Warner-Chilcott Scholarship in Medical Technology which I am, in effect, "unveiling" here today. It is specific; it is practical; it is a realistic step in meeting a far-reaching need. Speaking for myself, the development of this award has given me an opportunity to see how the principles, which the Commission on Health Careers applies impartially to all health professions, fit the particular need of a particular profession. Though it may be obvious, it is also reassuring to find that, like all good rules, they work both ways.

As President of Warner-Chilcott Laboratories, I have given a good deal of thought to industry perspectives on the health professions. I must say that all of them, including medical technology, look pretty much the same —whether one looks at them with the eyes of industry, or government, or voluntary health services and private practice.

At Warner-Chilcott, our business is to serve specific needs in specific health areas, and this kind of service for the field of medical technology is an important and growing part of our rapidly expanding pharmaceutical program. As you probably know, our Laboratory Supply Division includes medical technologists and allied laboratory people in its management and field positions; these people share the concern of your Society for the technical and educational and personnel needs of the medical technology profession. Our research, product development, marketing and advertising programs are structured around these needs, wherever possible.

These mutual interests represent the *where*, *why* and *how* of our new scholarship. It is made possible by an annual grant from the Laboratory Supply Division of Warner-Chilcott—to be administered by your Society, the American Society of Medical Technologists. Details have been worked out during the past year between Dr. Leanor Haley and your Executive Board, and Raphael Cohen, who heads this Warner-Chilcott Division.

I am glad to have this opportunity of expressing appreciation to Dr. Haley and to all of those who have given so much time and effort to creating a sound and constructive scholarship plan.

As a post-graduate scholarship to be awarded only to a practicing medical technologist for preparation to teach in this professional field, I think we can say that this grant is something of a trail-blazer—I under-

stand it is the first of its kind in medical technology. Without attempting to go into all the details which are listed in the Scholarship Brochure—I do want to point out some of its major specifications: the scholarship will hereafter be given annually; it is open to A.S.M.T. members who have worked for the past three years in a laboratory associated with an Approved School, or who are already teaching in an Approved School; and it may be used to begin or continue graduate studies toward a Master of Science degree in one of the medical sciences.

Though this is just one scholarship—a relatively modest grant for a highly specialized purpose—it has far-reaching implications:

—It is an expression of industry responsibility to help meet the educational and manpower needs of the health professions.

—It recognizes the increasing demand for scientific skills—the need to apply these skills in utilizing what is already known about health, and in maintaining the progress of the health sciences;

—It emphasizes the importance of assuring that the health field—your field in the broadest sense—has the teachers, the educational methods and tools, which new professional services and new students require.

—And it pays full tribute to the character and standards of professional work as cited in the Vannevar Bush definition.

These are just some of the meanings that can be read between the lines of this scholarship.

There are others which, in the long run, hold great promise. Look at it this way: Everyone of the pressures on health careers we have discussed today—including scientific progress, population change, competition for manpower—take their momentum from strength, not weakness. None of us—including the Commission on Health Careers—can change these pressures. Working together, what we can do is to capitalize on their strength:

—the strength of a growing population—with a great new generation of youth soon to be available for productive activity, and with untapped manpower resources in people of every age;

—the strength of scientific research—already pushing out beyond the laboratory to develop new health skills and to join with industries and communities in creating a healthier environment.

—the strength of competition—which has already brought our health professions together in this common effort to seek a fair share of the nation's available manpower.

On balance—weighing strengths against problems and pressures—we have as much cause for confidence as for concern. Not only in medical technology, but everywhere throughout the health field, there are signs that mark 1958 as a-year-with-a-turning point.

One of these is the continuing and whole-hearted cooperation with which the health professions are tackling the task ahead in Health Careers. Another is the new and broader alliance represented by the Commission on Health Careers. With the dedication and determination and enthusiasm which this audience so well exemplifies, the health professions will surmount the manpower challenge—and all the other challenges which, whatever else is uncertain, the future will surely bring.

STUDIES WITH SIMPLIFIED TESTS FOR URINE KETONES†

By NORA ALLEN, B.S., M.T. (ASCP),
MARTHA WINSTEAD, A.B., M.T. (ASCP), A. S. GIORDANO, M.D.††
South Bend Medical Foundation, South Bend, Indiana

Introduction

Tests for ketones have long been included in standard procedures for urinalysis but in most hospital laboratories such a test has not been a part of the "routine urinalysis."[1] Within recent years the detection of ketonuria has become simpler with the widespread use of a tablet test. Even greater simplicity appears to be offered with the introduction of a simple strip which is merely dipped into the urine to detect ketone bodies.

In order to evaluate the usefulness of this new simple strip test Ketostix*), studies have been made in which the strip test has been compared with the standard nitroprusside tablet test (Acetest**). The present report describes the results of such a comparison in a large series of urine received in two hospital laboratories. This report also describes the prevalence of ketonuria in random urine samples from hospital patients.

Methods

Strip Test. This test was carried out according to the procedure recommended which involves dipping the strip in the sample of urine and observing one minute after dipping for the presence or absence of a lavender color. With positive reactions the degree of the reaction is determined by comparing the color of the strip with the color chart.

The strip test consists of a stiff piece of special paper, the tip of which is coated with a mixture of sodium nitroprusside, glycine and sodium phosphate. The strip readily reacts with acetoacetic acid but in contrast to other nitroprusside tests does not react with acetone.

Tablet Method. This test was carried out by the method recommended in which one drop of urine is placed on the tablet and the presence or absence of a lavender color noted at 30 seconds. If the reaction is positive it is compared with a color chart which indicates the degree of the reaction.

The tablet is composed of sodium nitroprusside, glycine, disodium phosphate and lactose. The test is quite sensitive to acetoacetic acid and is somewhat less sensitive to acetone.

Handling of Urines. Tests for ketone bodies were carried out on the urines as soon as they were received in the laboratory. In the majority of instances the period between voiding and testing was quite short. However, in occasional cases there was a period of time between voiding and testing which might maximally be 12 to 15 hours.

Results

Observations on the reactivity of acetoacetic acid, acetone and beta-hydroxybutyric acid with the strip and the tablet were made by adding these three substances to normal urine. The acetoacetic acid was prepared by the procedure described by Hawk, Oser and Summerson[2] and

* Ketostix is a registered trademark of the Ames Company Inc., Elkhart, Ind.
** Acetest is a registered trademark of the Ames Company Inc., Elkhart, Ind.
† Read before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June 1958.
†† Dr. A. S. Giordano died February 15, 1958.

the beta-hydroxybutyric acid was a commercial sample (Aldrich Chemical Co., Inc.). Concentrations of acetoacetic acid, acetone and beta-hydroxbutyric acid in urine as shown in Table I were tested with the strip and the tablet. This tablet shows that the strip reacts only with acetoacetic acid in urine whereas the tablet is reactive with both acetoacetic acid and acetone. Neither of these tests reacts with beta-hydroxybutyric in urine. This nonreactivity with beta-hydroxbutyric acid is a common characteristic of all other nitroprusside and ferric chloride tests for ketone bodies.

TABLE I
Reactivity of Strip and Tablet Test

Constituent and Concentration	Tablet		Strip	
	Urine A	Urine B	Urine A	Urine B
Acetoacetic Acid				
100 mg. %	large	large	strong	strong
25 mg. %	moderate	moderate	moderate	moderate
10 mg. %	small	small	trace	trace
5 mg. %	negative	negative	negative	negative
Acetone				
1000 mg. %	strong	strong	negative	negative
200 mg. %	moderate	moderate	negative	negative
50 mg. %	trace	trace	negative	negative
10 mg. %	negative	negative	negative	negative
beta-Hydroxybutyric Acid				
1000 mg. %	negative	negative	negative	negative

Table II includes results obtained when the stick test and the tablet test were performed on approximately 4,000 random urines received in the two hospital laboratories. The color chart supplied with the stick test designates three grades of positive reactions as "small," "moderate," and "large." The color chart supplied with the tablet test also designates three grades but here they are called "trace," "moderate," and "strongly positive." In Table II results of each test have been reported in terms consistent with its particular color chart. Faint reactions with the strip or with the tablet in which the color was less than "small" on the one color chart or less than "trace" on the other color chart were recorded as negative. Results obtained with the stick test and the tablet test

TABLE II
Comparison of Tablet Test and Strip Test for Ketonuria
(Urines from Hospital Patients)

Acetest	Ketostix	Hospital A	Hospital B
negative	negative	2808	462
trace	negative	12	7
negative	small	5	4
trace	small	193	37
trace	moderate	3	1
moderate	small	16	9
moderate	moderate	116	14
moderate	large	7	2
strong	moderate	6	4
strong	large	112	31
Both positive (ungraded)		0	59
Totals			
Both tests positive		453	157
One positive, one negative		17	11
Both tests negative		2808	462
Total Urines Tests		3278	630

agreed very well. Almost invariably urines were either positive with both tests or were negative with both tests. Only 0.7% of the urine samples gave mixed positive and negative results. The stick test gave the positive result one-third of the time and the tablet test gave the positive result two-thirds of the time. In all these cases the positive reactions were only weakly positive.

When the data obtained from the two hospitals were tabulated, it was observed that hospital "A" had a 14.4% incidence of ketonuria in a series of 1295 urines and hospital "B" had a 23.3% incidence of ketonuria in its series of 387 urines. The validity of this high figure was determined by running a second series of urines making certain that results on all urines received in this laboratory were included in the tabulations. These tabulations substantiated the results obtained previously. In the second series hospital "A" had a 13.5% incidence of ketonuria among 1983 urines tested and hospital "B" a 27.6% incidence of ketonuria among 243 urines listed. These findings are summarized in Table III.

TABLE III
Incidence of Ketonuria in Random Urines from Hospital Patients

	No. of Positive Urines	Total Urines Tested	Incidence of Katonuria
Hospital A			
Series I	186	1295	14.4%
Series II	267	1983	13.5%
Hospital B			
Series I	90	387	23.3%
Series II	67	243	27.6%
Average (4 series)			19.7%

Discussion

The data obtained in the evaluation of the new strip test for ketone bodies indicate that it is a simple practical means for determining ketonuria. Its accuracy is comparable to the accuracy of the standard tablet test and its sensitivity appears to be of the same order of magnitude.

The comparability of behavior of the tablet and strip tests with routine hospital urines is particularly interesting in light of the fact that the strip does not react with acetone. Folin[3] indicates that the major constituent of ketotic urine is acetoacetic acid. Free and Free[4] reported that the color obtained in nitroprusside reactions of ketotic urine is essentially all due to acetoacetic acid. The present study of strip and tablet reactions with hospital urines has demonstrated the utility of the strip test even though it reacts only with acetoacetic acid.

The relatively high incidence of ketonuria in random hospital urines is quite impressive. Recognition that the inadequate food intake that is common in acute disease can account for ketosis makes the high incidence of ketonuria understandable. The frequency of ketosis in hospital patients provides a good basis for inclusion of a test of this sort as a part of a routine urinalysis. There is a reason to believe that clearcut recognition of the presence of ketosis might aid in the treatment of the patient.

Cook[5] has observed a 2.9% incidence of ketonuria in a series of 616 freshly voided urine samples presented for testing at a diabetes detection booth. These urines were from a heterogenous group which included healthy well nourished individuals, patients going to charity

clinics and poorly nourished individuals accompanying patients. The tests employed for ketonuria were the same as employed in the present study. In a separate study involving one urine sample from each of 125 healthy working adults this investigator found that none of the urines contained ketone bodies. Thus it appears that ketonuria is found rarely in healthy well nourished adults, may be found occasionally in patients seen by a physician, and is found frequently in acutely ill hospitalized patients.

Summary

A simple strip test called Ketostix for the detection of ketonuria has been compared with the standard nitroprusside tablet test, Acetest. The new strip test is accurate and has a sensitivity comparable to the standard tablet test. It is extremely simple to use. The strip test reacts readily with acetoacetic acid but does not react with acetone. Nonreactivity of the strip with acetone does not detract from the clinical utility of the test. In routine urines from hospital patients about 20 per cent of the samples contained ketone bodies.

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TECHNIQUE FOR MAKING BLOOD CULTURES ESPECIALLY FROM PATIENTS WITH CHRONIC FEBRILE ILLNESSES[1, 2]*

DEXTER H. HOWARD, Ph.D. and CHARLES M. CARPENTER, M.D.[3]

Department of Infectious Diseases
University of California School of Medicine, Los Angeles
and
Department of Pathology
Long Beach Veterans Administration Hospital, Long Beach, California

Among the laboratory procedures commonly employed in the definitive diagnosis of febrile syndromes, none is given more emphasis than a blood culture. Indeed, in certain types of infections such as brucellosis, bacterial endocarditis, and other bacteremias, a diagnosis which will lead to effective therapy is dependent upon the results of a bacteriologic examination of the patient's bloods. Yet it is precisely this procedure which too often fails to provide necessary information for the clinician. The transient nature of the bacteremia in many infectious diseases often precludes the recovery of the infectious agent in culture. Even though this phenomenon is an important consideration, numerous other factors may be responsible for the negative blood culture.

A survey by Engley, Page and Scott[7] revealed a lack of uniformity of methods in the bacteriologic examination of blood. In reviewing recommendations for carrying out blood cultures, the above authors noted that variations occurred with respect to type and amount of medium, containers, quantity of blood inoculated, atmospheric requirements, and period of incubation. Reasonable agreement prevailed that a temperature of 37°C be employed for incubation. Bennett[1] has recently reviewed the problem of "bacteremias" and has made valuable suggestions concerning the detection of the infectious agent. Standard text books such as those by Smith, *et al.*[19] and Todd and Sanford[20] recommend techniques designed for the isolation of the type of causative agent anticipated. A febrile episode is rarely well enough characterized so that the causative agent can be predicted. In addition, clinical laboratories are called up to make "routine" blood cultures with little or no information concerning the type of microorganism suspected. Furthermore, the indiscriminate therapeutic use of antibiotics prior to making a definitive diagnosis has, in the last decade, made it most difficult to isolate the infectious agent. Inasmuch as the clinical laboratory must select a method from a great diversity of recommended procedures, greater uniformity in the use of approved methods should furnish more dependable results.

The purpose of this report is to present data obtained by using several different blood culture techniques which in our experience have yielded the greatest number of positive cultures. The report deals with the following topics concerned with making satisfactory blood cultures: skin antiseptics, transportation of specimen to laboratory, media, interval of cultures, the Castaneda method, the lysed blood technique, incubation, blood cultures during antibiotic therapy, and the serum specimen.

Materials and Methods. Twenty ml. of blood was obtained after careful disinfection of the skin with iodine (2 percent) and alcohol (70 percent). Five ml. of blood was distributed to each of the following: (a) a

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flask containing 75 ml. trypticase-soy broth,* (b) a Castaneda bottle containing brucella broth and agar,** (c) a screw cap tube containing 20 ml. of sterile distilled water and (d) a clean sterile test tube.

The two media receiving whole blood were incubated at 36.5°C in an atmosphere of 10 percent carbon dioxide. The Castaneda bottle was subcultured simply by tipping the bottle to wash the bloodbroth mixture over the slant (see Results for more complete description and discussion of this procedure). The trypticase-soy broth was subcultured on 5 percent human blood agar plates on the third, sixth, ninth, fourteenth and twenty-first days of incubation. The lysed blood in the distilled water was centrifuged at 3,000 r.p.m. for 30 minutes and the supernatant was discarded. One-tenth ml. aliquots of the remaining blood sediment were inoculated onto brucella agar,** human blood agar, and chocolate agar, all of which were incubated at 36.5°C in an atmosphere of 10 percent carbon dioxide. The remaining sediment was inoculated into thioglycollate broth.*** Subcultures of the thioglycollate broth were made as they were with the trypticase-soy broth and were incubated under anaerobic and aerobic conditions.

The remaining 5 ml. of blood in the sterile test tube was allowed to clot, centrifuged, and the serum removed and stored at a —20°C.

Further details of procedure or technique will be discussed along with the results.

Results and Discussion. Skin Antiseptics. Thorough preparation of the skin with antiseptics is essential for a reliable blood culture. Price[15] in his discussion of surgical antiseptics has critically reviewed the literature on the relative effectiveness of various antiseptic agents. He points out that "one or two percent iodine dissolved in 70 percent alcohol is an excellent skin antiseptic." Price further shows that most of the agents which have been suggested as substitutes for iodine as skin antiseptics have proven unsatisfactory for one reason or another after more extensive observations. In our experience, iodine followed by 70 percent alcohol constitutes a most effective method of preparing the skin for venipuncture.

Transportation of Blood to the Laboratory. For best results blood should be inoculated directly into the media and in the studies herein reported media were always inoculated at the bedside. Since direct inoculation is not always possible, blood must be transported to the laboratory. Often the simplest technique is to allow the blood to clot, remove the serum when the specimen arrives in the laboratory and culture the clot after communition. Dubos[6] noted that successful isolations have frequently been reported from clotted blood submitted to the laboratory for serologic tests. Preparations of clots for inoculation of media without contamination is difficult, however. Glassware to be used in transportation of blood to laboratory must be scrupulously free from soaps and detergents, since these agents are highly toxic to microorganisms.

Anti-coagulants are sometimes employed during transportation of the specimen for culture. Reid[16] has shown that the anions citrate and oxalate which are commonly used as anti-coagulants are somewhat

* Baltimore Biological Laboratories, Baltimore, Maryland.
** Albimi Laboratories, Brooklyn, New York.
*** Difco Laboratories, Detroit, Michigan.

toxic for microorganisms. Therefore, exposure for several hours or days to either of these anions may adversely influence the result of the microbiologic examination.

Heparin, which is less toxic, is prohibitively expensive for routine use. Chelating agents employed as anti-coagulants in obtaining platelets from blood by Freeman[9] and by Dillard, Brecher and Cronkite[5] may be useful in preventing coagulation of blood to be used for cultures. However, such agents have not been studied to determine their usefulness in this regard.

Culture Media. Several dependable enriched broths and agars are now available commercially for routine blood cultures. Schuhardt, Rode, Foster, and Oglesby[15] and Pickett and Nelson[14] observed that certain lots of peptones are unsatisfactory for growth of stock strains of *Brucella* from small inocula. Such factors must be kept in mind in selecting a peptone suitable for use in blood cultures. Two media which have proven most satisfactory in our work are trypticase-soy broth* and brucella broth**. Huddleson[11] has recently shown that the addition of blood to media "elevates" the pH and that in isolating *Brucella* the medium should have an initial pH of 6.2 which after addition of from 2-5 ml of blood to 50-100 ml of broth becomes 6.8-7. The possibilty of a bacteremia due to an anaerobic or microaerophilic infectious agent necessitates the inclusion of thioglycolate broth with the aerobic culture media. Special media are required in the diagnosis of such diseases as tularemia and leptospirosis, (Foshap[8]; Hirschberg, Maddry, and Hines.[10]

Although the use of poured agar plates for blood cultures is still practiced in certain laboratories, Bennett[1] is of the opinion that the technique offers no advantage, is more "tedious and time consuming" and that information concerning the number of bacteria per ml of patient's blood derived from this source has limited value.

Interval for Multiple Cultures. It is generally accepted that in patients with intermittent chills and fever, the optimum time for obtaining a blood culture is from one or two hours prior to the expected rise in temperature. Inability to predict such an event necessitates the adoption of a more arbitrary schedule. During a recent study of blood cultures in chronic illness, Carpenter and his associates (2, 3) investigated the relationship of the time and number of bleedings as factors contributing to the successful isolation of the infectious agent. The results indicated that the chances of recovering an organism from a patient are directly proportionate to the number of blood cultures made. The maximum benefit of multiple cultures on a single day was attained by culturing at least two specimens on the same day. These results are in accord with the conclusions of Bennett (1). Thus in most circumstances from 2 to 4 cultures per day made at convenient intervals constituted an adequate procedure.

Although the advantage of anticipating the temperature rise is obvious, our experience and that reported in the literature (1) shows that if any arbitrary time sequence is to be followed, from 2 to 4 cultures daily on two successive days is reasonable and convenient timing for making blood cultures.

* Baltimore Biological Laboratories, Baltimore, Maryland.
** Albimi Laboratories, Brooklyn, New York.

The Castaneda Method. In 1947 Castaneda[4] recommended a procedure combining agar and broth in a bottle for the bacteriologic examination of blood for *Brucella*. Comparative studies have demonstrated this method to be equally effective when employed as a routine method of culturing blood for other infectious agents. The Castaneda bottle may be prepared in a variety of sizes and shapes. Essentially, the bottle contains an agar slant, the base of which is immersed in broth. The agar and broth bottle must be prepared in two stages. After the agar has been dispensed into the bottle and hardened, broth is added aseptically and the bottles are then incubated 24 hours to assure sterility. The amount of agar and broth employed depends on the size of the bottle to be prepared. An 8 ounce screw-capped bottle containing 40 ml. of agar and 65 to 70 ml. of broth is of convenient size when culturing 5 to 10 ml. of blood.

The incorporation of the agar slant with the broth in this manner provides an easy means of subculturing the broth without opening the bottle. Every two or three days bottles are tilted to wash the broth-blood mixture over the surface of the agar. Growth can be detected readily by observation of the slant. At the end of the incubation period bottles showing no growth may be discarded as negative thus eliminating much tedious subculture work. The obvious time saving advantage of the technique more than offsets the minor disadvantage of the two-stage preparation process.

Comparative studies were carried out with the Castaneda procedure and the more widely used trypticase-soy broth. The results obtained with the Castaneda method compare very favorably with the simple broth technique (Table 1 and 2). Inspite of the small positive isolations, the favorable outcome of the studies as well as the obvious technical advantages suggest that the Castaneda method is a valuable blood culture procedure.

The Lysed Blood Technique. Blood cultures employing hemolysed blood were first advocated by Rosenow[17] in 1914. In 1951 Pickett

Table 1

Positive Blood Cultures Obtained With the Castaneda and Trypticase-Soy Broth Procedures

Cultures	Castaneda Method* Number Positive	Trypticase-Soy Broth Number Positive
100	12	8**

* Castaneda Method employed Albimi's Brucella broth and agar.
** One culture not isolated by the Castaneda technique.

Table 2

Bacteria Isolated by the Castaneda and Trypticase-Soy Broth Techniques

Organism	Trypticase Soy Broth	Castaneda Method
1. Staphylococcus aureus		
a. Hemolytic	5	7
b. Non Hemolytic	1	3
2. Aerobacter aerogenes	2	2

and Nelson[14] modified the technique especially for the isolation of *Brucella* from blood. Because the latter procedure is complicated by many steps, the chances for contamination of the specimen are increased. In addition, the technique employs citrate as an anti-coagulant. These objections led Carpenter and his Associates[2,3] to modify the procedure by adding 5 ml. of blood directly to 20 ml. of sterile distilled water in a screw-capped tube. After thorough shaking, the specimen was centrifuged, the supernatant discarded and the concentrated sediment inoculated into a variety of media. The results obtained from 233 blood cultures, designed to compare the modified lysed blood technique and the Castaneda method, are presented in Tables 3 and 4. Seventeen positive blood cultures were obtained with Castaneda's[3,4] method and eight from culturing the sediment from lysed blood cells. Three of the microorganisms isolated from the "lysed blood" procedure were anaerobes, (Table 4) and were isolated undoubtedly because thioglycollate broth was employed as one of the media into which the blood sediment was cultured (Table 3). Thus it is our experience that the Castaneda procedure is superior to the others tested and it is highly recommended for use in routine blood cultures. Thioglycollate broth or some medium for anaerobic incubation should always be inoculated together with the aerobic Castaneda Bottle.

Table 3

Positive Blood Cultures Obtained With the Castaneda and Lysed Blood Procedures

Patients	Cultures	Castaneda Method		Sediment from Lysed Blood**	
		Positive		Positive	
		Number	Percent	Number	Percent
68.....	233	17*	7.4	8	3.5

* In two instances cultures were positive by both techniques.
** Mediae employed: Blood agar, "chocolate agar", Albimi's brucella agar, and thioglycollate broth.

Table 4

Bacteria Isolated by the Castaneda and Lysed Blood Procedures

	NUMBER	
	Castaneda Procedure	Lysed Blood Technique
1. **Staphylococcus aureus** (hemolytic).....	4	2
2. **Staphylococcus aureus** (nonhemolytic).....	6	1
3. Diphtheroids		
a. aerobic.....	3	2
b. anaerobic.....	..	2
4. **Gaffkyatetragena**.....	1	
5. **Brucella abortus**.....	1	
6. **Streptococcus viridans**.....	1	
7. Streptococcus, nonhemolytic, anaerobic.....		1
8. Unidentified gram negative rod.....	1	

Incubation. Reasonable agreement exists among most microbiologists that 37°C represents a satisfactory temperature of incubation for blood cultures. Inasmuch as the temperature of many small laboratory incubators frequently varies 0.5°C, a safer range is from 36°C to 36.5°C. Also, it has been shown that lower temperatures may result in the isolation of a greater

number of positive cultures (12). The duration of incubation prior to discarding is not standardized. Recommendations range from as few as five days (21) to as many as 4 to 6 weeks (1). A routine procedure of 14 to 21 days may be considered to be adequate in most instances. Under very special circumstances, such as culture for *Leptospira*, a longer incubation period of four to six weeks is essential.

Incubation under increased carbon dioxide tension is essential for the detection of certain species especially *Brucella abortus* and *Neisseria meningitidis*. From 5 to 10 percent of the air is replaced with carbon dioxide, or lighted candles are placed in a closed container with the cultures. The growth of aerobic pathogenic microorganisms is in no way impaired by incubation under carbon dioxide tension.

Blood Cultures during Antibiotic Therapy. The wide-spread application of antibiotic therapy often makes interpretation of a negative blood culture difficult if not impossible. Frequently the patient has been treated with an antibiotic before the blood culture was made. The use of penicillinase to inhibit the bactericidal effect of penicillin in blood, para-amino-benzoic acid to antagonize sulfa drugs, and cysteine to inhibit streptomycin have been practiced for some time (6). The use of such inhibitors is advisable when known antibiotic therapy includes any or all of the above drugs. Unfortunately, a large number of antibiotics are in current use for which, as yet, no antagonists or inhibitors are available. Clinicians should advise the microbiologist whenever the patient has had or is receiving antimicrobial therapy so that useful inhibitors, if available, may be incorporated into the culture media.

The work of Newman, Torres, and Guck[13] has indicated that antibiotic treatment does not always preclude the isolation of infectious agents. They observed that 20 of 26 patients with bacterial endocarditis treated before hospital admission yielded a positive culture and only 15 of 22 patients who had received no previous therapy gave positive blood cultures.

The Serum Specimen. The importance of obtaining paired specimens of serum in the course of a febrile disease cannot be overemphasized. The first specimen should be collected soon after the onset of illness, if possible, and a second specimen from 10 to 14 days later. Serum should be obtained early in the acute phase of the disease at the time of the primary blood culture. In febrile diseases of bacterial, viral, or fungal origin a rise in specific antibody titer is of primary importance. Thus, the results of serologic tests on serum taken early in the disease process may be compared with those obtained from a specimen taken at a later stage of the disease in order to detect a rise in titer. In the absence of a specimen taken during the early or acute phase of the disease, comparison is impossible, and valuable information is lost. Even if serologic studies are not anticipated, a serum specimen should always be taken and preserved for future reference.

Summary. Several techniques and methods for the bacteriologic examination of blood are described and recommended in an attempt to develop a uniformly dependable procedure which may be employed effectively in blood cultures. A brief summary of the procedures recommended is

set forth in Table 5. It is hoped that the discussion and recommendations will be helpful to microbiologists discouraged by the diversity of procedures recommended in the literature and dissatisfied with the results of their own techniques.

Table 5

Summary of Recommended Procedures for Routine Blood Cultures

Procedure	Recommendation
Skin disinfectant	Iodine and alcohol (70%)
Container	Castaneda bottle (8 oz.)
Media (amount)	Albimi brucella broth* (60-70 ml.) and Thioglycollate broth (10 ml.)
Amount of blood	5-10 ml.
Number of specimens	2/day for 2 succesive days
CO_2 tension	10%
Time and temperature	14-21 days—36.5° C.
Serum	Always taken and preserved.

* B.B.L. Trypticase-Soy Broth has also been used effectively. Two percent agar was added in preparation of the slants of the Castaneda bottle.

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THE POUR PLATE: A SIMPLIFIED TECHNIQUE IN ANTIBIOTIC SENSITIVITY TESTING*

JOHN SUTTON, B. S.
Bacteriology Dept. of Porter Hospital Sanitarium & Hospital, Denver, Colorado

The use of various methods and procedures in testing microbial susceptibility to antibiotics has been discussed by numerous investigators. Some of them include the agar well (British), the disc agar plate diffusion technique, serial tube dilution, a disc-tube method, and the seeded agar method of Mitchell. Each method is adequate for its purpose but some of them, notably the serial tube dilution technique, are not suitable for practical use because of the time and materials involved. The most popular technique available in the diagnostic laboratory is the agar (streak) diffusion method. It has been proven to be reliable, clinically accurate and efficient. The disadvantage of this technique lies in the fact that 6 to 12 hours are needed before the antibiotic susceptibility can be determined. A recent technique devised by Mitchell and his workers describes a hemoglobin indicator method employing the use of a blood agar plate overlaid with seeded nutrient agar. This technique allows for the determination of the susceptibility of the organism to a specific antibiotic within 2 to 4 hours. The only drawback to this method (in our experience) has been the additional use of materials (i.e., blood agar plate plus nutrient agar). In order to expedite time and save media as well, our laboratory has modified this procedure and has employed the use of the pour plate alone.

It is the purpose of this paper to compare the results of the standard streak plate agar diffusion method and the seeded agar hemoglobin indicator plate method of Mitchell with the modified pour plate technique used in our laboratory. The antibiotic sensitivity discs (Difco) which were used included Penicillin, Aureomycin, Tetracycline, Terramycin, Chloromycetin, Streptomycin (in 10 mg. concentrations) and penicillin (2 units).

Method: Mix specimen to be tested with 10-20 ml. of melted nutrient agar (Difco) cooled to 45° C. Pipette 1% sterile blood (discarded from blood bank). Pour into sterile petri plate and allow to solidify. Press discs of various antibiotics lightly upon media. Incubate at 37° C. Observe at half hour intervals. Zones appear about the specific antibiotic from 2-4 hours. The media changes from a cherry red to burgundy or purple representing a change or reduction in hemoglobin.

Interpretation: Reading may be interpreted as "resistant" (no zone of inhibition around a designated drug) or "sensitive" (definite zone of in-

Method Used	No. of Tests	Tests Agree	Tests Vary
1. Streak plate (agar-diffusion) Method	377	377	0
2. Blood agar plate with seeded top layer of nutrient (Mitchell's hemoglobin reduction)	377	350 (90%)	37 (10%)
3. Pour Plate: Seeded nutrient agar with blood added (hemoglobin reduction modification)	377	377	0

* Received for publication February, 1958.

hibition) to a particular antibiotic being used in much the same manner that readings are made in the agar diffusion method.

Summary: Two techniques for the determination of susceptibility of infectious materials to antibiotics are compared with a modified technique of the hemoglobin indicator method which is used routinely in our laboratory. The modified technique has the advantage not only of being both rapid and accurate but economical as well.

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Correction: This article was listed in error in the Table of Contents for the May-June issue.

VENIPUNCTURE—AN EVALUATION OF CERTAIN ASPECTS OF PSYCHOLOGY AND TECHNIQUE*

By BERNICE CULLEY, B.S., M.T. (ASCP)

105 East Columbia St., Evansville 11, Indiana

The casual reader may brush the subject of venipuncture aside as too basic in concept and too elementary to merit consideration. This erroneous assumption, so widespread among all branches of the medical profession, provides the stimulus for this evaluation.

Less attention is given to venipuncture technique in literature and in training schools than to any other laboratory procedure, yet it is the keystone upon which much of Medical Technology is built.

The few seconds spent in drawing blood often represent the technologists' sole patient contact, and therefore provide the basis for the attitude of the general public toward both technologists and technology. No matter how exactingly the subsequent testing is done, if the original phlebotomy is not skillfully and tactfully performed, the patient will be convinced the operator is ill-trained and incompetent, and will have little faith in the final report.

Often neophytes in technology are merely instructed in the application of the tourniquet, cautioned to "keep the bevel up," and sent forth to learn by experience. Unfortunately, the patient also learns by this —one could scarcely find less desirable advertising.

Much adverse publicity can be avoided if laboratory workers will apply a few simple suggestions to lessen the patient's discomfort. While these can be useful in dealing with hospital patients, they are primarily designed for the doctor's office, where the patient is usually less ill and more critical of the treatment he receives.

The first consideration is preparation for the patient before calling him into the room where the blood is to be drawn. Have all equipment laid out and ready. Do not keep the patient waiting unnecessarily; time spent waiting to be called into the laboratory is usually also spent in actively imagining the evils to come. By the same token nothing is more upsetting to a patient than to be forced to sit and watch the technologist assemble a gleaming array of tools, topped by a needle that suddenly looms disproportionately large.

Keep in mind that sights and smells to which you are accustomed may be highly offensive to lay people. Especially if there are urine or stool specimens standing around in the laboratory, it may be well to let the patient see you wash your hands—but do not prolong it. Make certain your hands and fingernails look clean. As you palpate for a vein, any grayness under the nails will stand out in stark contrast to the light skin of the patient's inner arm.

A pleasant smile and a cheerful word will do a great deal to ease the situation. If this is a new experience for the patient, take time to explain briefly and simply what you propose to do. NEVER tell a child OR an adult that it will not hurt. Be truthful; but do not exaggerate.

The patient's arm should be extended on a table or other firm support at a height that insures easy availability to the operator without undue discomfort to the patient. Apply the tourniquet snugly at least two or

* Received for publication April 1958.

more inches above the crease line, being careful not to pinch the skin. Rubber tubing, wide elastic, or a blood pressure cuff may be used, but the wider band is generally more comfortable to the patient.

Instructing him to open and close his fist several times not only aids distention of the vessels, but also provides a temporary distraction from the business at hand. The veins may be further encouraged to fill by gently massaging from wrist toward elbow, or by thumping lightly with a forefinger.

Take ample time to select the best vein, checking both arms. Almost any well supported vein is suitable, although the branches of the cephalic are most commonly employed. If neither of these is palpable, the accessory cephalic is often well anchored and visible. The median basilic should be attempted only as a last resort, and then only by careful and experienced personnel because it lies close to the intercutaneous nerve, and because too deep a thrust may pierce both walls of the vein and enter the brachial artery that lies beneath. Small superficial veins will often roll and may collapse easily, while very firm ropey vessels are sometimes thrombosed.

If the vessels are small and not easily seen, it is often advantageous to mark the course of the vein above and below the selected puncture site with washable ink. This eliminates the need for repalpation after entry.

Cleanse the site thoroughly with 70% alcohol. Many writers suggest iodine, but this scarcely seems necessary in routine phlebotomy, since some people suffer unpleasant reactions from its use.

The needle size is a matter of individual choice, although most authors recommend gauge 19 or 20. We have had consistently good results with a gauge 22, one inch, with standard bevel. This leaves no visible mark, while a 20 often leaves a small hole. In drawing quantities above 20 cc, a 21 or 20 gauge will be more satisfactory. Needless to say, a needle longer than one inch has a bad effect on the patient, and dull or burred needles are inexcusable.

Casually suggest to the patient that he focus his attention elsewhere, and talk with him as you perform the puncture. This not only diverts his attention, but his responses give you an early warning if he chances to be one of those unfortunate individuals subject to fainting.

Make the puncture well below the crease of the elbow in order to avoid unnecessary soreness later from repeated bending of the arm. Actual entry should be made at any angle of 45 degrees or less, with a short, decisive stroke through the skin while it is held taut by the thumb of the free hand. Care should be taken that thumb pressure is not so great as to flatten the vessel. Where the vein is prominent and well supported a single sliding stroke may be used to enter both skin and vessel, but it is preferable to use one thrust to penetrate the skin and a second to enter the side wall of the vein. This lessens the possibility of sliding past a rolling vessel into the deeper tissue.

The syringe may be held in any position affording complete control to the technologist while still permitting entry from an acute angle. We recommend the method which places the first two fingers and the thumb of one hand on the barrel rim, and the other two fingers on the plunger. This technique may seem awkward at first, but presents several ad-

vantages. Because aspiration is effected by pulling against the plunger with the third and fourth fingers, this is a one-handed venipuncture, with the other hand free to stabilize the arm, pull the tourniquet and hold the pledget. This is especially valuable in dealing with children. Since gentle suction actually begins with entry, blood appears in the syringe the instant the vein wall is pierced and the risk of passing through both walls is minimized. Novices may also avoid slipping out of the vessel by accident, as sometimes occurs when two-handed aspiration is employed.

Withdraw the necessary amount of blood with steady, even pressure on the plunger. Too much suction may cause patient discomfort, vein collapse and adherence of the vessel wall to the bevel, cutting off blood flow.

When sufficient blood has been obtained, brace the third and fourth fingers against the plunger and release the tourniquet, if this has not been done earlier. This helps prevent a backlash of blood into the vein as pressure is reduced.

Do not remove the needle so quickly as to cause pain, but apply a pledget firmly to the puncture site as soon as the needle is withdrawn. Failure to apply sufficient pressure, either by hand or by bending the arm, may result in a hematoma.

All bottles should be clearly labeled immediately to assure the patient that his blood will not be confused with that of another.

Especially where long sleeves of colored material are worn, the application of a small band-aid to the puncture site is a good precaution. Children's distaste for the procedure may be lessened by colored patches to the site, or even a small gift such as a toy balloon.

Adults who are unusually apprehensive and children (especially in the early teens group) should be allowed to lie down to have blood drawn, but no allusion should be made to fainting.

All of these suggestions come under the heading of good common sense. A little extra attention to small, seemingly insignificant details will pay off many times over in better public relations.

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A SIMPLIFIED UREA CLEARANCE TEST*

HAROLD V. CONNERTY, M.D., ANGLIS R. BRIGGS, B.S., AND EDWARD H. EATON, JR.

Department of Pathology, Veterans Administration Hospital, Wilmington, Delaware

Kidney function is primarily concerned with the elimination of metabolites; in this way, the constancy of the "milieu interieur" is maintained. Renal function tests, consequently, must measure the excretory ability of the kidneys which is reflected in their ability to form dilute or concentrated urine and to maintain normal blood levels of the nitrogenous metabolites. One of the most sensitive tests of renal function which is able to detect even slight degrees of excretory impairment, provided it is properly performed, is the Urea Clearance Test (Ref. 5, 7, 8). Urea Clearance connotes the number of milliliters of blood completely cleared of urea per minute; i.e., the number of milliliters of plasma which contains the amount of urea removed per minute by renal excretion (Ref. 2).

The greater the degree of renal impairment, the smaller the number of milliliters of blood cleared of urea per minute. This indicates a decrease of excretory efficiency. One important factor which must be considered in the performance of the Urea Clearance Test is the fact that the rate of elimination of urea is influenced to some extent by the rate of urine flow. When the rate of urine flow exceeds 2 ml. per minute, the rate of elimination of urea is directly proportional to the blood urea content (Maximum Clearance). With urine volumes below this figure, the rate of urea elimination is proportional to the square root of the urine volume (Standard Clearance) (Ref. 2).

The Maximum Clearance as a measure of renal function is more reliable than the Standard Clearance and many authors stress the importance of maintaining adequate diuresis during the period that the urine is being collected so that the Maximum Clearance can be determined (Ref. 1, 5, 8).

Use of the Urea Clearance Test has been restricted because it requires the cooperation of nurse and patient and because it involves a determination of urinary urea, a troublesome procedure necessitating a simultaneous determination or removal of the urinary ammonia (Ref. 6). The authors have devised a simple accurate specific method of determining urinary urea by direct Nesslerization, a procedure which has heretofore been impractical due to interference from interfering nonspecific chromogens. These are eliminated by the use of iodine in the manner described below. This is an adaptation of the use of iodine as proposed by the authors in their method of determining blood urea nitrogen (Ref. 3), and preformed urinary ammonia nitrogen by direct Nesslerization (Ref. 4).

Method

Two entire samples of urine collected over consecutive or successive accurately-timed intervals of 30 to 60 minutes each (in order to calcu-

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This article was reviewed and is published with the approval of the Veterans Administration The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion of the Veterans Administration. Dr. Connerty is Chief of Laboratory Service, Mr. Briggs is Biochemist, and Mr. Eaton is Chemistry Technician.

late the output of urine per minute) and one specimen of blood drawn at the mid-interval are necessary. One hour before the collection of the urine is begun, the subject should drink two, preferably three, glasses of water, in order to secure a urine output greater than 2 ml. per minute so that the more accurate Maximum Clearance may be calculated. Restriction of meals is not necessary*, and the specimens may be collected at any convenient time, although several authors state that the best time is between breakfast and lunch (Ref. 2, 10, 11). The blood sample** is analyzed for urea nitrogen by the procedure (Ref. 3) described by the authors in a previous publication. 1 ml. of blood is treated with urease and the proteins are then precipitated with 9 ml. of tungstic acid solution. To 1 ml. of the filtrate, one drop of 2% iodine solution, 9 ml. of water, and 1 ml. of Nessler's solution are added. The optical density is determined at 450 mu against a reagent blank, and the B.U.N. calculated. Each urine sample is also analyzed for urea nitrogen by the authors' direct Nesslerization method as follows:

Reagents:—

Urease Solution: Dissolve 10 grams of urease powder (obtainable from Sigma Chemical Company, St. Louis 13, Missouri) in 50 ml. distilled water, and add 50 ml. of C.P. glycerine. Mix.

M/15 Phosphate Buffer, pH 7.2 Mix 27 ml. of M/15 Monobasic Potassium Phosphate (9.078 gm. KH_2PO_4 dissolved in 1000 ml. of water) with 73 ml. of M/15 dibasic sodium phosphate (11.876 gm. of Na_2HPO_4. 12 H_2O dissolved in 1000 ml. of water).

2% Iodine Solution: Dissolve 3 grams of potassium iodide and 2 grams of elemental iodine in 15 ml. of water, then dilute to 100 ml. with distilled water.

Nessler's Solution (Vanselow—Ref. 11). Dissolve 34.9 gm. of potassium iodide in 50 ml. of distilled water. Add 45.5 gm. of red mercuric iodide and agitate until solution is complete. In a separate flask, dissolve 112 gm. of potassium hydroxide in 200 ml. of distilled water. After cooling, mix the two solutions and dilute with distilled water to 1000 ml.

Nitrogen Stock Standard: Dissolve 4.720 grams of dry reagent-grade ammonium sulfate in water, add a few drops of concentrated sulfuric acid, transfer quantitatively to a liter volumetric flask and dilute to volume with distilled water.

1ml. contains 1mg. of nitrogen.

Nitrogen Standard: (1 ml. = 20 micrograms of Nitrogen)

Transfer 2 ml. of Stock Nitrogen Standard to a 100 ml. volumetric flask and dilute to volume with distilled water.

Procedure—Urinary Urea:

Into a 100 ml. volumetric flask place 0.5 ml. of urine, 1 ml. of M/15 phosphate buffer, 5 ml. of water and 4 drops of 10% solution of urease. Mix and allow to stand for 20 minutes. Then dilute to the 100 ml. mark with water. Mix. Transfer 1 ml. of this incubated diluted urine to a 10 ml. graduate or cuvette and add one drop (0.05 ml.) of 2% iodine solution followed by 9 ml. of water. Then add 1 ml. of Nessler's solution and mix. The Optical Density of this read at 450 mu (blue filter) against a reagent blank composed of 1 ml. of Nessler's solution, 1 drop

* Some authors interdict the use of coffee and tea because caffeine increases the Urea Clearance.
** Theoretically plasma should be used, but in the Urea Clearance Test it makes no difference.

of 2% iodine solution, and 10 ml. of water gives the *Urea Nitrogen plus Ammonia Nitrogen*. The ammonia nitrogen value, which must be subtracted, is determined as follows: Due to the 1 to 200 dilution of the urine and the salutary effect of iodine, this value is usually very low, of the order of 0.01 Optical Density. Place 0.5 ml. of the urine in a 100 ml. volumetric flask and dilute to the 100 ml. mark with distilled water. Transfer 1 ml. of this diluted urine to a 10 ml. graduate, or cuvette, add 1 drop (0.05 ml.) of 2% iodine solution and 9 ml. of water. Then add 1 ml. of Nessler's solution and mix. Read the Optical Density at 450 mu against the reagent blank described above and subtract this value from the Optical Density of the Urea Nitrogen plus Ammonia Nitrogen to get the true Optical Density of the Urea Nitrogen. Or one may use the above Nesslerized preparation as the blank for reading the Urea Nitrogen plus Ammonia Nitrogen and thus cancel out the Optical Density of the Ammonia Nitrogen directly in the photometer.

We stress again the desirability of having minute volumes of urine greater than 2 ml. so that only Maximum Clearances are being dealt with. A standard is not really necessary, as will be shown later, but if one should wish to prepare one, proceed as follows: Place 20 micrograms (conveniently contained in 1 ml.) of Ammonium Nitrogen in a cuvette, add 1 drop of 2% iodine solution, and sufficient water to make a volume of 10 ml. Then add 1 ml. of Nessler's solution and mix.

Calculation:

1 ml. of blood filtrate representing 0.1 ml. of blood is Nesslerized at a volume of 11 ml. and 0.005 ml. of urine is also Nesslerized at a volume of 11 ml.

$$\text{The Blood Urea Nitrogen} = \frac{\text{O.D. Bld}}{\text{O.D. Std.}} \times Q \times 10 = \text{ug BUN/ml} \qquad \text{Eq. (1)}$$

$$\text{The Urine Urea N} = \frac{\text{O.D. Urine}}{\text{O.D. Std.}} \times Q \times 200 = \text{ug Urine Urea N/ml} \qquad \text{Eq. (2)}$$

Dividing equation 2 by equation 1, we get

$$\frac{\dfrac{\text{O.D. Urine}}{\text{O.D. Std.}} \times Q \times 200}{\dfrac{\text{O.D. Bld}}{\text{O.D. Std.}} \times Q \times 10} = \frac{20 \times (\text{O.D. Urine})}{\text{O.D. Blood}} = \frac{U}{B}$$

where O.D. = optical density
Std. = Standard
Bld. = Blood
Q = Concentration of the Std. in micrograms
V = Urine volume in ml. per minute
U = Urine Urea Concentration
B = Blood Urea Concentration
ug = Micrograms

$$\text{Maximum Clearance} = \frac{U}{B} V$$

Therefore Maximum Clearance =

$$\frac{U}{B} V = \frac{20 \times \text{O.D. Urine}}{\text{O.D. Blood}} \times V$$

Consequently, no standard is really necessary. The ratio of the optical density of the urine to the optical density of the blood multiplied by the

factor 20 and by the number of ml. of urine excreted per minute gives the required answer. The values of Clearance obtained from the two urine specimens should agree with each other within 10%; otherwise, the results of the test should be considered unreliable. The mean normal Maximum Clearance is 75 (75 ml. of blood cleared of urea per minute).

The percent of average normal Maximum Clearance $= \frac{100\ UV}{75B} = \frac{1.33\ UV}{B}$

Correction for body size is required in the case of children, the factor being $\frac{1.73}{\text{body area in sq. meters}}$ The formula for the percent of average normal Maximum Clearance then becomes:

$$\frac{1.33\ UV}{B} \times \frac{1.73}{\text{body area in sq. meters}}$$

The surface area may be obtained from tables used in calculating basal metabolic rates.

Sources of Error: The most important source of error is caused by inaccurate determination of the number of ml. of urine excreted per minute. This is caused by incomplete emptying of the bladder, by inaccurate timing of the intervals during which the urine specimens are collected, or by failure to deliver complete urine specimens to the laboratory. Decomposition of the urine prior to analysis must also be prevented.

It may not be remiss to explain the rationale of the general equation for calculating clearance: $\frac{UV}{P}$, where U equals the concentration of the substance in the urine, P equals the concentration of the substance in the plasma, and V equals the volume of urine formed per minute. Let M represent the substance whose clearance is being determined.

$$\frac{U}{P} = \frac{\text{mg M per 100 ml. urine}}{\text{mg M per 100 ml. plasma}} = \frac{\text{mg M per ml. of urine}}{\text{mg M per ml. of plasma}} = \text{the number}$$

of times the amount of M in 1 ml. of urine exceeds the amount of M in 1 ml. of blood plasma. This quantity multiplied by the number of ml. of urine excreted per minute gives the number of times that the total quantity of M excreted per minute in the urine exceeds the amount of M in 1 ml. of blood plasma. For example, if the quantity of M excreted per minute is 60 times that in 1 ml. of plasma, then 60 ml. of blood plasma contain the amount of M which is excreted in the urine per minute; i.e., 60 ml. of blood plasma are cleared of M per minute. This is the definition of Clearance.

Summary

We have simplified the performance of the Urea Clearance Test from the laboratory standpoint by developing a simple, accurate method for determining urinary urea, based upon the neutralizing effect of iodine upon interfering urinary chromogens and upon the stabilizing effect of iodine upon Nesslerized solutions. We have stressed the desirability of dealing with only maximum clearances. The rationale of the formula for calculating renal clearances has been discussed.

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PROPOSAL FOR A CERTIFIED STANDARD FOR USE IN HEMOGLOBINOMETRY—SECOND AND FINAL REPORT*

Prepared by National Academy of Sciences, Division of Medical Sciences, Washington, D. C.

In 1953 the Hematology Study Section of the National Institutes of Health requested the Division of Medical Sciences of the National Academy of Sciences—National Research Council to explore the possibility of establishing a hemoglobin standard for general use throughout the country. In response to this request, the Division in 1954 organized an Ad Hoc Panel for the Establishment of a Hemoglobin Standard under its Subcommittee on Blood and Related Problems.

Dr. George Cartwright, School of Medicine of the University of Utah, accepted the Chairmanship of the Panel. Its members were Dr. David L. Drabkin, University of Pennsylvania Graduate School of Medicine; Lt. Col. William H. Crosby, Jr., Walter Reed Army Institute of Research; Dr. George Brecher, National Institutes of Health; Dr. Wallace Brode, National Bureau of Standards; Dr. Israel Davidsohn, Mt. Sinai Hospital, Chicago, representing both the College of American Pathologists and the American Society of Clinical Pathologists; and Dr. A. H. Neufeld, representing the National Research Council of Canada. In 1956 Mr. John Gould succeeded Dr. Brode, and in May, 1957, Dr. Bradley E. Copeland, New England Deaconess Hospital, Boston, and Dr. Donald Brown of the Hackensack General Hospital succeeded Dr. Davidsohn as representatives of the College of American Pathologists and the American Society of Clinical Pathologists, respectively. The Panel was also fortunate in having the cooperation of Dr. E. J. King of the Postgraduate Medical School of London, who has given his full support to this undertaking.

Most routine methods of clinical hemoglobinometry depend upon the photometric measurement of a blood sample after quantitative conversion of the hemoglobin which it contains into one or another of its derivatives. For the standardization of such a procedure there is needed a color standard, which, when measured in the photometer and cuvette in routine use, will establish the relation between instrument reading and concentration of the particular hemoglobin derivative. This relation can then be used in converting instrument readings for unknown samples of blood to concentration of hemoglobin by the use of Beer's law or by construction of a calibration curve.

The problem, however, is complicated by the fact that several methods of analysis involving conversion to different forms of hemoglobin are in common use. Each of these methods should have its own standard. Since a multiplicity of standards is neither desirable nor practicable, the Panel decided, early in its deliberations, to select only one method of analysis for which a suitable direct standard would be developed. It should, however, be pointed out that this procedure does not preclude the use of the standard for the indirect calibration of another method. All that need be done is to construct a calibration curve based on the measurement of a small series of *normal* blood samples by the standardized method and also by the method which the analyst prefers to adopt for routine use.

In the opinion of the Panel, the most significant contribution which

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could be made to the refinement of clinical hemoglobinometry would be the widespread adoption of a single method of analysis. Failing this, the indirect calibration of other methods with the chosen method offers the only simple photometric means for the comparison of data. The procedure, however, is subject to error if the blood sample contains significant amounts of certain of the abnormal forms of hemoglobin. For example, methemoglobin and carbon monoxide-hemoglobin are quantitatively convertible to cyanmethemoglobin, but not to oxyhemoglobin.

The Panel reviewed the several photometric methods in current use and came to the conclusion that the procedure involving the measurement of hemoglobin as cyanmethemoglobin was the most promising. It offered the following advantages:

1. A simple and accurate procedure has been devised by Drabkin involving the addition of a single reagent to the sample of blood; [13, 14]
2. The method has been adopted by the U. S. Army after extensive field trials;[12]
3. All forms of hemoglobin likely to occur in circulating blood, with the exception of sulfhemoglobin, are determined by the method;
4. The color is suitable for measurement in filter-type photometers as well as in narrow band spectrophotometers because its absorption band in the region of 540 mμ is broad and relatively flat; and
5. The U. S. Army has had extensive experience in the use of solutions of cyanmethemoglobin as direct standards and has found these to be satisfactory.[12] The U. S. Army standards have remained unchanged in optical density for extended periods when stored at refrigerator temperatures provided bacterial contamination was avoided.

On the basis of this evidence, the Panel decided to develop a certified solution of cyanmethemoglobin as a standard, and to promote an extensive field trial of its suitability.* A preliminary report of its recommendations and plans appeared in a number of scientific and technical journals in 1955.[1-9] This report outlined recommendations of the Panel, described arrangements for the preparation and distribution of certified standard solutions of cyanmethemoglobin, and invited cooperation in an extensive field trial of the use of the standards and of the recommended method of analysis.

More than a thousand laboratories volunteered to cooperate in the trial sponsored by the National Research Council. Distribution of the standards was made with the assistance of the College of American Pathologists, the National Association of Clinical Laboratories, the Walter Reed Army Institute of Research, and the National Research Council of Canada. The laboratories not only received descriptions of the procedures for the use of the standards to calibrate photometers, but also directions for the routine determination of hemoglobin in the form of cyanmethemoglobin.

* Proteins are not generally regarded as highly stable in dilute solution, even if sterile. In spite of the experience of the U. S. Army the Panel hesitated to adopt a standard to which theoretical objection might be taken and explored other more stable materials. These included colored glasses and solutions of pigments so prepared as to approximate the absorption by cyanmethemoglobin of the spectral transmission of the filters, prisms or gratings employed in photometers and spectrophotometers. These alternatives were rejected because (a) no suitable mixture of pigments suggested itself, (b) it would be necessary to provide a series of glass standards to match the sizes and shapes of commonly used cuvettes, and (c) for some instruments the glasses would also have to match the lens effect of the round cuvettes and their contents.

The results of the study were most gratifying. The need for, and the ready and grateful acceptance of, a simple method for the standardization of hemoglobinometers was apparent. The recommended method of analysis was likewise well received. At the onset of the field trial study only seven per cent of the cooperating laboratories had been determining hemoglobin as cyanmethemoglobin. At the time of the last report, two-thirds of the cooperating laboratories were using this method.

In the first field trial, the standard solutions were prepared from crystalline hemoglobin by Dr. David Drabkin. Three solutions in carefully determined concentrations of approximately 60, 40 and 20 mg. of hemoglobin per 100 ml. were distributed. The optical densities of the final solutions were independently confirmed and a continuing control on stability was maintained in the laboratories of Dr. Brecher, Mr. Gould, Col. Crosby, Dr. King and Dr. Neufeld. Agreement having been reached on the optical density values, the hemoglobin concentrations of the standards in mg. per cent were computed from the optical densities, assuming that the extinction coefficient of cyanmethemoglobin per milligram-atom of iron (55.85 mg.) per liter is 11.5 and that the pigment contains 0.335 per cent of iron.

Two problems were encountered during the course of the field trial study. The first of these was the growth of certain micro-organisms observed in some samples in spite of the presence of cyanide. This made it necessary to prepare and to maintain the solutions under sterile conditions. The second problem was a change, unpredictable in degree and not reported by all checking laboratories, of two to six per cent in the optical density six to nine months after distribution of the standard. Samples from each lot were found to have undergone varying degrees of change, mostly fading, which was compensated in some samples by a comparable increase in turbidity. The standards prepared and distributed by the U. S. Army in its earlier field trial had remained unchanged in optical density for three years. Inasmuch as these solutions had been prepared directly from whole blood or from washed red cells, it was suspected that the manipulation involved in the preparation of the crystalline hemoglobin for the National Research Council standards might have reduced the stability of the pigment. Therefore, a new standard was prepared from washed cells. The new standard was further modified by increasing the concentration of cyanide, since some previous preparations with such higher concentrations had shown greater stability and since the growth of most organisms would be limited by the higher concentration of cyanide.

Since the percentage change in optical density of the first standards was greater with increasing dilution of the hemoglobin pigment, and since cyanmethemoglobin solution follow Beer's law, the second standard was distributed in only the most concentrated (60 mg. per cent of hemoglobin) of the three dilutions.

The second group of standards, modified as outlined above, was distributed in July and August of 1956. Their stability was determined in the laboratories of Dr. Drabkin, Dr. Brecher, Mr. Gould, and Col. Crosby. The stability was satisfactory for at least nine months from the time of preparation, no change of more than two per cent in optical density being observed.

The members of the Panel have concluded that solutions of cyanmethemoglobin, when prepared, calibrated and handled properly, are acceptable as standards for hemoglobinometry. They recognize that such standards are not ideal in all respects. However, until better standards can be developed, they are of the opinion that the availability of this reagent will greatly simplify the calibration of hemoglobinometers and will greatly increase the accuracy of hemoglobinometry over previously employed practices.

Finally, they encourage further independent investigation in the hope that an even better standard may be developed, particularly one with improved stability and more certain maintenance of sterility.

The National Research Council supplies of the standard cyanmethemoglobin solution are now exhausted and no further production is planned under the auspices of the Academy-Research Council. However, standards are now available from several commercial sources. The NAS-NRC has recommended the establishment of a program of certification of commercially produced cyanmethemoglobin standards to determine conformance with the specifications it has established. In response to the need for the establishment of such a program, as defined by the NAS-NRC, the College of American Pathologists has arranged for certification through the facilities of the laboratory of the American Medical Association in Chicago. On the basis of data obtained through this laboratory, the College of American Pathologists will certify whether commercially produced standards which have been submitted comply with the specifications established by the NAS-NRC. *All users are urged to insist that the cyanmethemoglobin standards they purchase commercially carry the certification label of the College.**

Detailed instructions for the preparation of the standards have been published by Crosby.[10-11] Producers of the standard or instrument manufacturers may obtain technical details on the adaptation to and use of the standard in the various hemoglobinometers by writing to the Division of Medical Sciences of the National Research Council.

The final recommendations of the National Research Council Ad Hoc Panel on the Establishment of a Hemoglobin Standard are as follows:

1. That cyanmethemoglobin be adopted as a standard in clinical hemoglobinometry.

2. That the standard be characterized spectrophotometrically on the basis that the extinction coefficient of one milligram atom of iron (c = 1 mg. atom of iron per liter, d = 1 cm.) in the form of cyanmethemoglobin at a wave length of 540 mu μ is 11.5.

3. That 0.338 per cent (w/w) be accepted as the iron content of hemoglobin (molecular weight of 16,5$\overline{20}$ per gram atom of iron) in accordance with the recent recommendation of the Protein Commission of the International Union of Pure and Applied Chemistry, and that a factor of 1,65$\overline{2}$ be used in calculating hemoglobin in mg. per 100 ml. from millimoles per liter.

4. That the standard be distributed as a single concentration of not less than 55 mg. of cyanmethemoglobin per 100 ml.

* In Canada discussions are now taking place in order to make one national laboratory responsible for the production and certification of the standard solutions.

5. That solutions be distributed in brown glass containers and in sterile condition.

6. For the present it is recommended that solutions should be used as standards for a period not to exceed nine months from the time of preparation. This dating period is based upon the results of the National Research Council field trial. As experience accumulates with commercially prepared samples, an extension of the dating period may well be found to be justifiable.

7. That the standard be prepared from either crystalline hemoglobin or washed erythrocytes.

8. That commercial producers of the standards submit representative specimens from each lot to the College of American Pathologists, Prudential Plaza, Chicago 1, Illinois, for certification:

a. That the concentration of cyanmethemoglobin is within ±2 per cent of the value stated on the label;

b. That the solution is substantially optically clear; and

c. That it is microbiologically sterile.

R. Keith Cannan, D.Sc.
Chairman, Division of Medical Sciences
National Academy of Sciences—National Research Council
Washington, D. C.

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COOMBS INHIBITION TEST*

By YVONNE DUPES, B.A., M.T. (ASCP)

St. Agnes Hospital, Fond du Lac, Wis.

Since the recognition of agammaglobulinemia with its manifestation of repeated bacterial infections, there has existed a need for an accurate, yet relatively rapid, method for the determination of the gamma globulin level in human serum which would be within the scope of the average hospital laboratory. The methods most widely used at the present time are those based on chemical, immuno-chemical, or electrophoretic procedures. The available chemical methods are time consuming and of questionable accuracy unless adequately controlled. The immuno-chemical methods, although highly specific, would seem to be unwieldly for the hospital laboratory. The electrophoretic methods require apparatus which is not always financially feasible. Gitlin and Janeway[3] point out that electrophoresis cannot give the accuracy needed in the determination of small concentrations of gamma globulin in human serum. There is some justification in the use of isohemagglutinin titers as an indication of the gamma globulin level, but correlation does not always exist and the method could not be used for an individual of the AB blood group.

In 1949, Wiener, Hyman, and Handman[5] published a relatively simple method for the determination of gamma globulin concentration. It is based upon a modification of the Coombs anti-globulin technique[1] and utilizes the most sensitive of all reactions, an antibody-antigen complex. The procedure requires materials already on hand in any clinical laboratory.

The Coombs anti-globulin technique for the detection of univalent antibodies can be summarized as follows:

1. When human serum containing univalent antibodies comes in contact in a saline medium with human red cells having the specific antigen, the antibodies will coat the cells.
2. All traces of free human globulin, which has the ability to inactivate anti-human globulin (Coombs) serum are removed by repeated washings of the cells with saline.
3. Coombs serum is added and causes the coated cells to agglutinate, thereby demonstrating the presence of univalent antibodies.

The Coombs inhibition procedure is based in principle upon the second step in the anti-globulin technique as described above. Any free human gamma globulin in the system has the ability to inactivate or inhibit Coombs serum, rendering it ineffective in the agglutination of coated cells. It follows that the presence of gamma globulin can be shown by using sensitized human red blood cells, human serum, and Coombs serum. If gamma globulin is not present in the human serum, the Coombs serum will cause agglutination of the sensitized cells, while if gamma globulin is present there will be inactivation of the Coombs serum and no agglutination of the cells. In serum containing an unknown amount of gamma globulin, it is possible to determine the amount present in terms of a percentage of normal by testing the ability of serial dilutions of the serum to inhibit the Coombs serum. The ability of normal serum to inhibit Coombs serum is used in parallel as a control.

* 1st Serology SPF Award 1958. Read before the 26th Annual Convention of the ASMT, Milwaukee, Wisconsin, June 1958.

Our attention was first called to the Coombs inhibition procedure by the work of Sturgeon and Brubaker[4] in which the principle of the test and case histories relating to its use were discussed. As the 1949 paper of Wiener and associates[5] was not available to us at that time,[9] a technique for the procedure was developed from Sturgeon and Brubaker's statement of principle. The technique as it evolved in our laboratory differs in several details from that described by Wiener, but not in essence. The procedure as well as a report of our experience with the test is presented.

Procedure

Materials

1. Sensitized Human Red Blood Cells—
   Fresh human red cells belonging to Group O, Rh positive are coated with univalent Rh_o antibodies and stored at —20 C until needed for use:
   a. Prepare a 2 per cent suspension of washed Group O, Rh_o positive red cells in physiological saline solution.
   b. Add an equal volume of serum containing univalent (blocking) antibodies (Rh_o slide typing sera may be used). To attain maximum coating of the cells, the Rh_o antibody titer of the serum should be well in excess of 1:16 by the albumin titration method.[6]
   c. Incubate anti-serum-cell mixture for thirty minutes in a 37 C water bath.
   d. To remove free gamma globulin, wash cells at least four times with large volumes of physiological saline, as in the Coombs anti-globulin procedure.
   e. Prepare sensitized cells for storage according to the technique of Dunsford and Bowley:[2]
      1) Centrifuge and remove supernatant fluid.
      2) Agitate tube continuously and add, in small aliquots, an equal volume of glycerol-citrate solution prepared by mixing four volumes of glycerin with six volumes of 5 per cent aqueous trisodium citrate solution.
      3) Divide into half ml aliquots and store at —20 C.
   f. Frozen cells which keep their reactivity for at least six months, are prepared for use when needed.[2]
      1) Cells are thawed at 37 C, centrifuged, and supernatant fluid removed.
      2) Add a volume of 12 per cent aqueous trisodium citrate solution equal to packed cell volume and let stand for ten minutes.
      3) Centrifuge and remove supernatant fluid.
      4) Wash cells with physiological saline until supernatant fluid is clear (usually 4 to 6 times).
      5) Dilute packed cell volume sufficiently with physiological saline to prepare a 2 per cent suspension.
2. Normal Serum Control—
   a. A large number of human serum samples are pooled. A sufficient number of determinations are done on this pool to verify that the gamma globulin concentration falls within normal limits (1:128 to 1:512 inhibition titer).
   b. Half ml aliquots are stored at —20 C until needed for use.

Test Proper

1. Serum dilutions—
   a. Two series of ten small test tubes are set up for each serum to be tested, and two series of ten tubes for the normal serum control.
   b. Add 0.15 ml physiological saline to first tube in each series, and 0.10 ml to each of remaining tubes.
   c. Add 0.05 ml of cell-free serum to the first tube of appropriate series.
   d. Mix and transfer 0.1 ml from tube one to tube two. Continue transferring serially, discarding 0.1 ml from tube ten. Use a clean pipette between each dilution or rinse original pipette for each serum in a large volume of saline between each dilution.
   e. Serum dilutions range from 1:4 in tube one to 1:2048 in tube ten.
2. Add one drop anti-human globulin (Coombs) serum to each tube. Shake gently to mix.
3. Add 0.1 ml of 2 per cent sensitized red cell suspension. Shake gently to mix.
4. Centrifuge at 1000 rpm for one minute.
5. Observe for inhibition of agglutination. The reciprocal of highest serum dilution inhibiting agglutination is the COOMBS INHIBITION TITER (CIT).
6. Results are reported as percentage of normal serum control. Divide titer of unknown by normal titer.

Experimental Results

A series of Coombs inhibition determinations were done in conjunction with isohemagglutinin titrations in order to establish the range within which normal results would fall. Some representative results appear in Table I, A through J. It can be seen that the greatest percentage of these have the inhibition titer of 1:256, which was the result consistently achieved with the pooled serum control. These results agree with those of Wiener, 1955,[7] 1957.[8] Table I, K through O, shows a series of determinations done on pretransfusion serum samples of hospitalized individuals receiving multiple transfusions. The last two determinations (P and Q) were done on fresh serum samples from an untreated individual. The time lapse between the two determinations was three months.

TABLE I

INHIBITION TITER	Blood Group	Anti-A Titer	Anti-B Titer
A 1:256	A	.....	1:32
B 1:256	AB	.....	.....
C 1:256	O	1:64	1:128
D 1:128	A	.....	1:128
E 1:512	AB	.....	.....
F 1:256	A	.....	1:32
G 1:128	O	1:32	1:8
H 1:256	A	.....	1:32
I 1:256	O	1:256	1:32
J 1:256	AB	.....	.....
K 1:64	O	1:128	1:64
L 1:64	A	.....	1:128
M 1:32	O	1:64	1:32
N 1:32	A	.....	1:16
O 1:64	A	.....	1:16
P 1:64	O	1:32	1:16
Q 1:64	O	1:16	1:16

Table II shows determinations done on a 6 year old boy, who, because of recurrent infections, was suspected of being a case of agammaglobulinemia. Three days after the first determination (A) he received a series of gamma globulin injections totaling 2.6 gm. The second determination (B) was done 2 days after the last injection of the series, and the third (C), 21 days after the last injection of the series.

TABLE II

INHIBITION TITER	Blood Group	Anti-B Titer	Percentage of Normal
A 1:32	A	1:4	13
B 1:256		1:8	100
C 1:64		1:16	25

Discussion

In our work with this procedure we have utilized frozen, maximally sensitized red cells from only one donor in order to keep the cell factor constant. An obvious advantage also is that the cells are on hand when needed, eliminating the time which would be consumed by sensitizing cells before each determination.

The use of a pooled serum containing an average normal amount of gamma globulin circumvents the necessity of searching for a "normal" for each test, keeps the normal value constant, and, most important, serves as a control for the Coombs serum and sensitized cells. It has been our experience that the frozen pooled serum consistently gives values identical to those obtained when the pool was first tested.

The inhibition titer given by a true normal serum is largely dependent upon the antibody titer of the Coombs serum which is used. The higher the titer, the more gamma globulin is needed for inhibition. Conversely, if the titer of the Coombs serum is low, less gamma globulin will be needed for inhibition, giving a higher inhibition titer. This indicates the necessity of a normal serum control.

Duplicate titrations are employed for the recognition of any introduced error. The duplicate titrations agree exactly if scrupulously clean glassware has been employed and good technique maintained.

Since the inhibition titer shown by various concentrations of gamma globulin depends upon (1) the titer of the Coombs serum employed, (2) whether or not maximum cell sensitization has been achieved, and (3) the technique employed, it is not possible to set up inflexible normal values. However, in consideration of the apparent correlation of the results cited by Wiener, 1955,[7] 1957,[8] and those obtained by us, it would seem that a normal inhibition titer would range between 1:128 and 1:512, or between 50 to 200 per cent of the normal control. Although in our experience with the test we have encountered no cases of agammaglobulinemia, Wiener, 1955,[7] gives data on two cases in which the inhibition titers were negative (no inhibition) and 1:2, respectively. Sturgeon and Brubaker[4] cite one case of agammaglobulinemia in which inhibition was partial at 1:2, and one case of hypogammaglobulinemia in which inhibition was complete at 1:8 and partial at 1:128.

Summary

1. The Coombs Inhibition Test as it is used in our laboratory is presented.
2. Based upon an antibody-antigen reaction, the determinations are of a highly specific nature.
3. Experimental results are presented to show the reproducibility of the procedure and to give a basis for the establishment of normal values.

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AN UNMASTERED ROLE WITH A CHALLENGE—* THE TEACHING SUPERVISOR

MARY FRANCES JAMES, A.B., M.T. (ASCP)
Teaching Supervisor and Ass't Professor of Medical Technology, University of Mississippi School of Medicine, Jackson, Miss.

The staff of every Approved School of Medical Technology must include a teaching supervisor in addition to the laboratory director. This is a requirement, according to the "Essentials of an Acceptable School of Medical Technology,"[1] adopted by the Council on Medical Education and Hospitals of the American Medical Association.

What is this position, considered important enough to be specifically required? The conceptions of the requirements for and responsibilities of such a staff member are surprisingly varied, frequently shockingly limited and even erroneous. This marked diversity is due, primarily, to the fact that ideas have been formed from observation of these positions as they are filled at the present time. Any composite picture of this role, even from broad experience in widely separated localities, will still contain marked discrepancies.

Why is there a confused picture in regard to the part played by the teaching supervisor in medical technology training? In general, the answer is that in many instances the potentialities of the role have not been recognized, resulting in much less than maximum utilization of technologists in these positions as well as only partial fulfillment by the teaching supervisors. There are some schools in which this title has been bestowed on one of the laboratory staff, solely to fill the space on the annual questionnaire.

Why do these positions vary so from school to school? There are many reasons for this wide variation which may be classed in three broad groups. First, are those due to the physical or organizational differences in the training centers. Second, are those resulting from varying degrees of understanding, interest and cooperation on the part of the school directors and hospital administrators. Third, are the reasons dependent on differences in the ability, initiative and dedication of the teaching supervisors themselves.

Wide variation can be expected on the basis of the divergent types of Approved Schools alone. There are training programs in large private clinics and small hospitals approved to train no more than two students per year and others in 3000-5000 bed hospitals with 40-50 students. Some teaching laboratories consist of a single area in which all types of procedures are performed; while others are completely departmentalized and associated with specialized units under the supervision of experts in that particular field. These basic differences—size, type of center, number of students and, departmental arrangement will cause considerable variation in the requirements for a teaching supervisor.

Other factors which differ among schools and affect greatly the teaching supervisor's duties are the number and caliber of the staff. The ideal staff is one composed of enough competent personnel to handle the laboratory work as well as an adequate number of experi-

* Read before the 26th ASMT Convention, Milwaukee, Wisconsin, June 1958.

enced instructors able to assist in teaching both the technical and theoretical aspects of the curriculum. Unfortunately, most schools function with much less than the ideal.

In most schools the teaching supervisor has time-consuming responsibilities in addition to those related to the training program. Many are head technologists in the clinical laboratory with the administrative details of that position. Others may supervise a laboratory section carrying a heavy load of routine work. It is the balance between designated and available time for the training program, and other duties which determines in large measure the effectiveness of the teaching supervisor.

Probably the most decisive factor in determining the extent to which a qualified teaching supervisor will be properly utilized in his assignment is—the attitude of the school director and the administration toward this position and the training program. It must be realized that the teaching supervisor does not replace the director in any way. The position is an adjunct to that of the directorship and should be appreciated for its value in relieving these very busy individuals of some of the burdens which otherwise would fall upon them. It should be considered a position of responsibility with specific responsibility delegated. Confidence should be placed in the person holding the appointment as evidenced by frequent consultations; joint planning; sharing of problems and visions; with encouragement in attempts to introduce new and progressive features into the training program. Adequate time must be alloted the teaching supervisor for performing the many functions of his position. The position of teaching supervisor cannot be considered a "sideline." The director also must give staunch backing to his staff in support of the training program, particularly in the many approved schools in hospitals unaccustomed to such educational programs. The necessity for additional highly trained staff, equipment and space for the conduct of a successful educational project, may not be understood by administrations for which medical technology training is a new hospital function.

Regardless of differences that exist from school to school, in the demands on the teaching supervisor, there are still similar basic responsibilities and an unlimited number of avenues for development toward successful fulfillment of this position in each of its various aspects. Only a qualified person can be expected to carry the many diversified responsibilities which may fall on a teaching supervisor. By discussing some of these responsibilities, the necessary qualifications will become obvious.

A teaching supervisor in a School of Medical Technology should function as a combination medical technologist, teacher, administrator, registrar, personal counselor, efficiency expert, public relations officer and professional representative. What position includes more fields thus offering greater challenge?

Before discussing the potentials of each phase of the role, it is necessary to start with the premise that every teaching supervisor is a dedicated, capable *medical technologist*. It is to be assumed that these appointments are given to individuals because of a better than average

scientific, academic background and, evidence of efficiency during extensive experience as a medical technologist.

Most teaching supervisors serve as *teachers* without benefit of any or but few college courses in Education. Such courses would be helpful but the following may be substituted, effectively: better mastery of the material being taught through constant reading and studying; attendance at seminars and refresher courses to keep informed; willingness to impart with patience whatever information one possesses; continual effort to develop good instructional techniques; close liaison with the clinical staff and, an awareness of general trends and innovations in Medical Education.

An overall program of instruction must be definitely planned ahead and rigidly followed. This does not mean that a program once planned is followed habitually from year to year. It should be revised to include new material or to meet special needs of a majority of the students as they become evident. Disappointment is frequently encountered because of deficiencies in the collegiate background of the students. It may fall upon the instructors to fill in the "gaps" during the hospital training period. A few review sessions in basic quantitative analysis, human anatomy or physiology; mimeographed material on the derivation of medical terminology, or supervised study sessions are examples of a few simple ways to meet particular needs of the students.

A teaching supervisor should be resourceful in obtaining and collecting teaching aids and materials which prove helpful to both the teacher and the student. If assistance is needed in learning to make good examinations, Montgomery's[2] treatise on this subject can be used. Lehman[3] has assembled valuable suggestions for preserving and making simulated samples of abnormal urine and cerebrospinal fluid for teaching purposes. Photomicrography is an excellent means of obtaining teaching slides from abnormal microscopic material as it is found. Such slides are more permanent than microscopic slides and preserved specimen but do not replace the need for collection of these materials. Movies are available on loan from a number of sources such as the Communicable Disease Center, Chamblee, Georgia and pharmaceutical houses. A list of visual aids can be obtained through the Education Committee of the A.S.M.T. The new Histology teaching movie now being prepared, should fill a long felt need and it is hoped will be only the first of many such teaching films. On the market are sets of Kodachrome slides as well as wall charts and manuals on a variety of subjects. Loan sets on parasitology and hematology can be borrowed from the Executive office of the American Society of Medical Technologists.

Other important teaching responsibilities are the compilation of a manual of procedures being used in the hospital laboratory; encouragement to read from reading lists furnished, based on a well rounded library which is kept up-to-date and, arranging for the attendance of students at seminars, rounds, conferences and special demonstrations.

One very essential teaching responsibility is not to the student technologists but to the graduate technologists who are called upon to instruct without prior preparation along this line. Hundreds of technologists are placed in this situation every year. Since learning to

instruct in Medical Technology is entirely an "on the job" process at the present time, "practice teaching" should be encouraged by giving the new instructor opportunity to prepare and present lectures; compile examinations; lead discussions and set up demonstrations. Every such attempt should be supported by encouragement and suggestions from the more experienced personnel, such as the teaching supervisor. Duties of this type accepted by the novice willingly and conscientiously carried out, will prove of inestimable value.

The *administrative* duties usually include handling of correspondence, such as replying to requests for information regarding the school. This task may be simplified by the preparation of an attractive information bulletin for mailing along with a brief personal note. Having mimeographed grade sheets, application forms, health records, etc., is also a time saver. Such forms must be formulated for the needs of each school. Helpful suggestions for these may be found in a booklet published by the Board of Schools of Medical Technology.[10]

Application materials must be assembled for consideration by the Admissions Committee or individuals who select the students. Following acceptance, there are numerous steps (some routine; others individual) in preparation for arrival of the new students. Worthy applicants may require financial help. Can assistance be given in finding a source? If housing is not furnished, are listings kept on available quarters? The dietary department must know when new students are to receive meals; the laundry be informed of additions being added to those eligible for that service and the business office notified regarding tuition owed or scholarships to be paid.

The scheduling of students should be done in a precise manner in regard to length of time in each section but not necessarily to the order of rotation. For the welfare of the students, there are a number of factors that may be taken into consideration. It is not advisable to rotate the same students together through a number of assignments. A better, more aggressive student may receive more attention from the instructor unconsciously, or by comparison, cause unjust evaluation of the capabilities of the weaker or more retiring student. It is also possible that a slow student might set a pace of accomplishment which would retard a better student. Separation of close personal friends as well as clashing personalities, when detected, may add greatly to the quality of work of both students involved. Month to month scheduling as far as possible has advantage over any rigid rotation schedule. For example, this serves to avoid assigning students to an era where the regular instructor is on vacation; there are new staff members not yet orientated themselves or temporarily understaffed.

As *registrar*, the teaching supervisor keeps the students' records which are required by the Council.[11] The grades, however, should not only be recorded and filed but evaluated for use in counseling and in planning methods of instruction according to the needs of individual students. Types of useful information which can be elicited by study of these records: whether or not the student is meeting the standard of performance expected on the basis of his college record; whether the student is markedly better in practical application than in mastery of

theoretical material or vice versa; whether he is improving after becoming orientated to hospital environment or not; whether there is evidence of need for additional instruction in a particular section or unusual aptitude displayed in one field warranting encouragement toward specialization.

Also by evaluation of grades, one important criterion for selection of students can become more standardized. Grades from different schools are rarely comparable. However, after study of the achievement of a number of students from various colleges over a period of time, it is frequently possible to judge the relative value of grades as given by the schools.

Counseling on academic matters is of prime importance but it must be realized that there are many personal problems which affect seriously, in many instances, the students performance. Homesickness, illness or other problems in the family; unsuitable living arrangements; being in love or breaking up of a love affair are a few of the personal situations which a student may be experiencing. Some of these difficulties might be improved with understanding help; some could be resolved only by the individuals involved; while knowledge of others might serve only to account for poor or declining quality of work. But whether there is a solution to the situation or not, it is a satisfaction to the student to know that there is someone with whom they can share freely such matters and receive in return thoughtful consideration and understanding.

An *efficiency expert* is one who determines whether the best means are being used to produce successfully a product of top quality. There are a number of simple ways to evaluate the effectiveness of a medical technology training program as an efficiency expert would.

The attendance at all formal sessions and spending of a period in each practical assignment by some one person permits determining whether the curriculum is complete in regard to coverage of material, well balanced between theoretical and practical instruction and, wisely divided in regard to time designated for each subject.

A mimeographed student check sheet devised for each section is valuable to both the student and staff. By use of these, the student has before him a list of the procedures which it is his responsibility to learn and which the instructor is expected to present—if not through an opportunity for practice, by discussion, demonstration or reading assignments. Of equal value is the use of a compilation of records from these student check sheets to determine whether adequate practice and instruction in the various procedures is being given in each section. Study of the monthly laboratory report on procedures performed gives similar information regarding the frequency with which various tests are made. On the basis of such data, the need for supplemental teaching material in certain areas may become evident.

Although the ability of the graduates to pass the Registry examination is not a foolproof criterion for judging the effectiveness of a practical training program, it does offer an objective basis for evaluation, particularly with the "IBM" reports as now made. The overall success of the instruction given by the school can be estimated as well as some idea obtained regarding the efficiency of teaching as done within each

division. Such information should result in attempts to correct any obvious deficiencies.

Because Schools of Medical Technology function within an integral unit of the hospital, it is necessary for the teaching supervisor to maintain good *public relations* with affiliated hospital services and, at the same time to help with assimilation of this educational program within a busy laboratory primarily responsible for service to patients. There are numerous relationships which must be supervised, for example, student to professional hospital staff; student to patient; student to other hospital personnel. By requiring the students to wear Approved School "patches" (obtainable from the Board of Registry), they are identified *as students* to personnel concerned and simultaneously publicize the fact that the School of Medical Technology exists. There is mutual benefit in close cooperation between the administrations of training programs in the hospital (e.g. X-ray, Physical Therapy, Nursing) because of similar problems.

Outside of the hospital the most important relationship is to the faculty and administration of the institutions of higher learning from which students are drawn. Close cooperation through interchange of visits and frank discussions should serve to strengthen both the preparatory education of the student medical technologists and the hospital training program. Recruitment of students can be done at the same time through talks and showing of the recruitment movies, "Career: Medical Technologist" and "The Human Cell and the Cyto-Technologist", to science clubs and classes. Such groups should be encouraged to visit the hospital on "field trips". Similar recruitment activities at the high school level are equally necessary.

Affiliation between colleges, universities and hospital training schools, if kept strong, will be of untold value. Another association which can prove to be a great asset to smaller schools, in particular, is one with other training schools in the vicinity. By collaborating in the presentation of formal sessions, a better lecture series can be presented through pooling the talents of the best qualified instructors from the cooperating institutions. At the same time, limited staffs are relieved of a part of their teaching load.

At all times, the teaching supervisor as well as all other Medical Technologists are *professional representatives.* Is this representation what it should be? Few, if any, students graduating from approved schools would fail to take the Registry examination if properly impressed during training with the idea that being an A.S.C.P registered technologist is an accomplishment of which one can be proud. Affiliating with their professional societies at the local, state and national levels as active members, upon successful registration, should be an event anticipated with pleasure by the students. This will be the case, if their instructors have demonstrated interest and satisfaction while working in these organizations and, receiving the benefits of attendance at seminars, workshops and other educational meetings sponsored by them.

There should be one aim common to all individuals holding the title of teaching supervisor—that of making available to an increased number of student technologists the best preparation possible for a career in medical technology. However, attempts to reach such goals should

not be made alone as they are unattainable without the team work of the director, teaching supervisor and laboratory staff showing mutual respect for the contributions of each other.

It is hoped that this analysis of the role of a teaching supervisor in an Approved School of Medical Technology may bring awareness of the unlimited possibilities of it and, that the few suggestions made may be helpful. The potentialities of the position should be a challenge to technologists, having pride in the profession of Medical Technology and recognizing that the future of their chosen profession is dependent on the quality of technologists produced by Approved Schools.

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THE IVY BLEEDING TIME*

ANN BELL, B.A.

Instructor in Medicine, University of Tennessee College of Medicine, Department of Medical Laboratories, Memphis, Tennessee

When laboratory procedures to predict the tendency to bleed at the time of surgery are indicated, the most important single test is the bleeding time. In a recently published article Dr. L. W. Diggs stated that, in patients scheduled for operation on whom special hemorrhagic studies are indicated, the determination of the bleeding time by the Ivy method should always be included.[2] In the detection and differential diagnosis of hemorrhagic disease, the determination of the bleeding time is most important. In the book *Hemorrhagic Diseases* by Drs. M. Stefanini and William Dameshek, the bleeding time is included in a series of "screening tests" that should be performed in the laboratory investigation of the bleeding patient.[7]

What is meant by the bleeding time? The bleeding time is that time required for effective hemostasis to occur following infliction of a standardized wound of the superficial blood vessels of the skin.

What happens normally when the skin is injured? When small blood vessels and capillaries are punctured, the pressure in the vessels is lowered by the escaping blood. The walls of the blood vessels constrict and the lumens of the vessels become smaller. Fluids containing thromboplastin and other powerful clot-promoting extracts mix with the outpouring blood when tissues are injured. The thrombocytes adhere to an injured vessel and wound margins, and they produce a blockage. Fibrin strands begin to form and soon there is a solid clot. The flow of blood stops because the clot is of sufficient strength to exceed that of the pressure in the vessel. In normal persons the skin is elastic and also aids in closing the wound.[6]

In the skin bleeding time multiple factors are involved. The blood vessels, the thrombocytes, and the tissues play an important role in stopping the flow of blood following trauma. The character of the clot is also important, for a feeble, non-retractile, and poorly adherent clot is incapable of closing the wound adequately and is swept away by the flowing blood as soon as it is formed. As a rule, the plasma components (fibrinogen, prothrombin, and accelerator factors) are minor factors in the production of an abnormal bleeding time. Defects in these components are usually compensated for by the vascular, tissue and thrombocyte factors.

There are three procedures which have been recommended for estimation of the bleeding time: 1) puncture of the tip of the finger, 2) puncture of the ear lobe (Duke method), and 3) puncture of the skin of the forearm (Ivy method).

The finger-tip puncture is the most commonly used method, but it is the least reliable. It is not recommended, for it may reveal no abnormality where there is one. The skin of the finger is elastic and thick, and it closes readily over the wound and promptly stops the flow of blood. In the past, the performance of the finger-tip bleeding time was an accepted and standard practice. It afforded the laboratory

* Presented before Tennessee State Society of Medical Technologists, Memphis, Tennessee, May 3, 1958.

director, the surgeon, and the hospital administrator a certain degree of protection, which comes from performing a customary procedure. However, because of present knowledge about laboratory tests for hemorrhagic diseases, such a procedure as the bleeding time by the finger-tip method will not be accepted, according to Dr. L. W. Diggs.[1,2]

The puncture of the ear lobe was first described by Dr. W. W. Duke in 1910,[3] and it gives a more reliable test than puncturing the finger. The skin of the ear lobe is thin, and the tissue is quite vascular. The ear lobe is relatively inaccessible, especially if the patient is in bed. The ear is flexible and soft, and it is difficult to gauge the depth of the stick. It is not easy to control bleeding if it is prolonged. This method does not allow two tests to be performed simultaneously. The results are not easily reproduced. The Duke method does not involve the stress of increased vascular pressure as does the Ivy method. Often the Duke method gives a shorter bleeding time than the Ivy method and abnormalities may not be revealed. However, the lobe of the ear may be the preferred site of puncture in infants and in uncooperative children.

The Ivy method of puncturing the skin of the forearm is the recommended procedure for the skin bleeding time. This method was introduced in 1935 by Dr. A. C. Ivy, Professor of Physiology at Northwestern University Medical School. Dr. Ivy suggested the skin of the forearm near the elbow as a puncture site. He also said that a sphygmomanometer around the arm above the elbow with the pressure at 40 mm of mercury should be used, in order to cut off effectively the venous return. In normal individuals he found little difference between this method and the Duke method. However, in a number of patients with jaundice, it was found that often when the bleeding time by the Duke method was normal, the bleeding time by the Ivy method was definitely prolonged. Therefore, Dr. Ivy felt that the venous pressure bleeding time would aid him in predicting the tendency of patients with jaundice to bleed following surgery.[4] In 1941 he suggested the use of a lancet with a controlled depth of 3 mm.[5] The Ivy method for the bleeding time has been modified in the Department of Medical Laboratories by the use of a disposable lancet which has a depth of approximately 4 mm.

Procedure

In performing the skin bleeding time, place a blood pressure cuff around the arm above the elbow and maintain a pressure of 40 mm mercury throughout the procedure. Clean the anterior surface of the forearm below the elbow with alcohol and allow it to dry. Select an area away from any visible blood vessels and distal to the antecubital fossa. Tense the skin at the proposed puncture site by grasping the posterior portion of the patient's arm between the thumb and the fingers of the left hand. Pierce the skin to a depth of 4 mm with a Hemolet or equivalent. Observe the time or start a stopwatch. Absorb drops of blood with filter paper at 30-second intervals until the bleeding has ceased. Whatman filter paper #1 is an ideal absorbent material. (Fig. 1.)

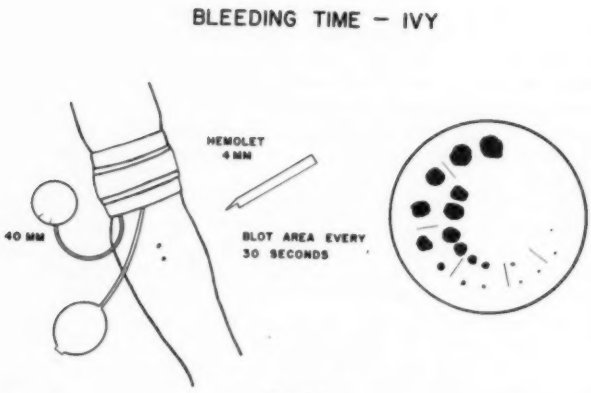

Figure 1
Ivy bleeding time using a disposable lancet. (Courtesy of Miller, Seward E., "Textbook of Clinical Pathology," 5th Edition, Baltimore, The Williams & Wilkins Co., 1955)

In a normal individual the first two or three drops of blood may be of small size. This is due to vasoconstriction which follows vascular injury. The drops grow larger for several minutes and finally reduce in size as hemostasis occurs. One or more of the drops of blood from the wound should be approximately 0.5 cm and preferably 1 cm in width. If there is no profuse bleeding at one minute (or 2 minutes), a second control puncture is made adjacent to the first stick. The drops of blood from both punctures are absorbed on the same piece of filter paper at the same time. (Fig. 2). The results of both tests are reported. The filter paper containing the absorbed drops should be saved and attached to the patient's record for future reference.

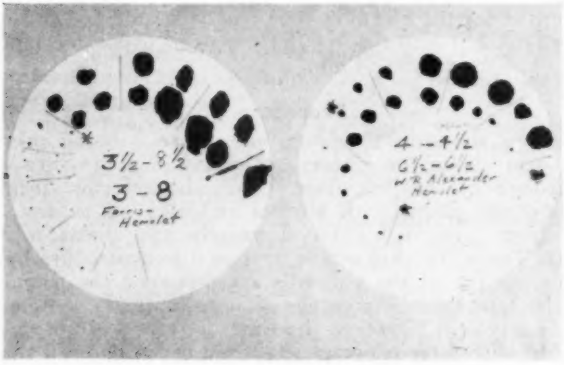

Figure 2
Normal bleeding times, Ivy method.

A sharp end point of the Ivy bleeding time is not always obtained, for in many persons there is often oozing of blood-tinged fluid from the puncture site for 1 to 5 minutes or more after the frank bleeding has stopped and the size of the drops has greatly decreased. Since normal individuals, as well as patients with hemorrhagic abnormalities, may have a prolonged oozing time, it is recommended that the bleeding time reported on the patient's record be the time of frank, free bleeding. It is not necessary to report the oozing time, since its clinical value has not been established.

Normal Values

The free flow of blood varies from 1 to 6 minutes. A bleeding time between 6 and 10 minutes indicates mild abnormality. A bleeding time above 10 minutes is decidedly pathological. (Figs. 3, 4.)

The Ivy bleeding time was performed on 363 medical students. The range of frank bleeding was from 1 minute to 6 minutes on 353 students (97%) with a mean of 3 minutes and 30 seconds. There were 11 students in the group who showed frank bleeding from 6 to 9 minutes, 30 seconds. In this same group of students 219 (60%) showed oozing of blood from one minute to 9 minutes and 30 seconds.

Important Observations

A disposable lancet is an ideal instrument, for it is made with a guard which gives a constant depth of puncture. It is inexpensive and is packaged in a sterile container. A Bard-Parker blade #11 may also be used, but it is difficult to control the depth of the puncture. Usually the bleeding time is longer with a Bard-Parker blade than with a non-surgical blade.

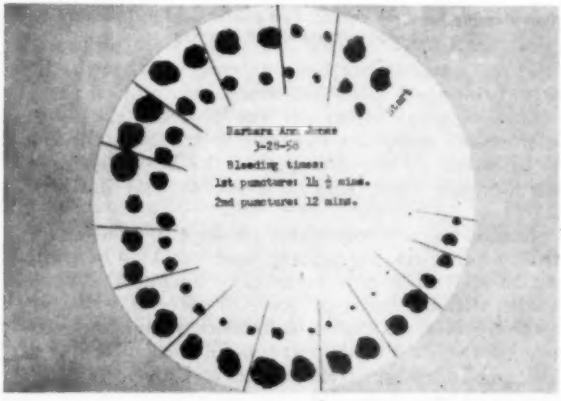

Figure 3
Prolonged bleeding time, Ivy method.

If the arm becomes cyanotic or if a patient experiences pain during the test as a result of venous stasis, the pressure in the cuff should be released at intervals. If numerous petechiae or ecchymoses appear in the skin, then venous stasis should be discontinued. If there is profuse bleeding, suggesting that a vein near the skin was pierced, the cuff pressure should be lowered. The technologist should apply a pressure bandage to this site for several minutes.

The test should be performed in a warm room, because superficial skin vasoconstriction due to cold air may give a short bleeding time.

If there is no sign of cessation of bleeding at the end of 15 minutes and the drops of blood have continued to be approximately the same size for several minutes, the test should be terminated. (Fig. 4)

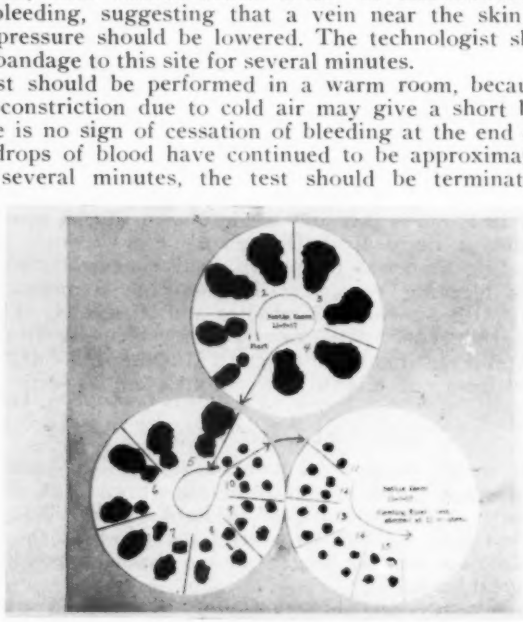

Figure 4
Abnormal bleeding time, Ivy method. Test discontinued at 15 minutes with no apparent cessation of bleeding.

Continuing the test after it has been determined that the bleeding time is significantly prolonged adds no more information. It also has an unfavorable psychological effect on the patient. Bleeding is stopped by placing a pressure compress over the wound. On the report a statement should be made to the effect that the test was discontinued at a certain time because there was no evidence that the bleeding would stop. (Fig. 3.)

If there should be a recurrence of bleeding after a temporary cessation, this finding is significant and should be noted. This is probably due to defective clot formation during the period of vasoconstriction with dislodgement of the clot after the vessel walls relax.

Frequently there is a noticeable difference in bleeding time obtained after puncture of the skin with a lancet in two different areas. There may be a prolonged bleeding time in one test and a failure to correlate with the second stick. In this instance one should repeat the bleeding time on the other arm and repeat the test on another day. All results should be reported on the laboratory data sheet.

If there is profuse bleeding with cessation in the normal period of time, this is considered a good test of hemostasis.

The bleeding time may vary at different periods of time. The test may be abnormal on one occasion and normal several weeks or months later. In the evaluation of the patient's problem, all tests must be included.[2]

Interpretation

In systemic vascular diseases, such as allergic purpura and hereditary capillary fragility, the bleeding time will be prolonged. It may be prolonged in purpura simplex, senile purpura, scurvy, and purpura secondary to infections and toxic states.[6] In addition, in vascular abnormalities in which there is a superimposed thrombocyte deficiency, such as idiopathic thrombocytopenic purpura, aplastic anemia, and leukemia, the bleeding time will be prolonged.

The skin bleeding time is prolonged in those conditions in which there is a thrombocyte deficiency. The bleeding time and the platelet count are not closely correlated. It is possible to have a prolonged bleeding time with normal platelets or a normal bleeding time with a decrease in thrombocytes. However, as a rule, when thrombocytes are less than 100 per 100 oil immersion fields on a blood smear, or less than 100,000 per cu mm, it is expected that the skin bleeding time will be prolonged.

When plasma thromboplastin components (AHG, PTC, PTA) are deficient, the bleeding time is usually normal due to the compensating effect of tissue thromboplastin, together with normal vascular mechanisms and thrombocytes. In diseases involving the first phase of coagulation, the bleeding time may be prolonged if a deep puncture of the skin is made with a surgical blade, for in this case the compensating mechanisms are inadequate.

In severe prothrombin deficiencies and in deficiencies of stable and labile accelerator factors, the bleeding time is variable but it is usually found to be normal.

A short bleeding time indicates normal hemostatic mechanisms.

Summary

In the bleeding time multiple factors are involved, the major ones being the blood vessels, thrombocytes, tissues and clot character.

The Ivy method of using a disposable needle with a guard, inflating a blood pressure cuff to 40 mm mercury, making two pucture wounds in the skin of the forearm and absorbing drops of blood from both punctures on filter paper is recommended as the procedure of choice for the skin bleeding time.

If the arm becomes cyanotic, if a hemotoma or numerous petechiae appear, if the patient experiences pain, or if there is profuse bleeding, the test should be discontinued. If there is no cessation of bleeding at the end of 15 minutes, the test should be terminated. A recurrence of bleeding after a temporary cessation is significant. When there are differences in bleeding times performed on the same date or on different occasions, all results should be considered in the evaluation of the hemorrhagic problem.

The finger-tip puncture is not recommended, for the elastic skin of the finger closes readily over the wound and may reveal no abnormality when there is one. The Duke method of puncturing the ear lobe is satisfactory and may be used in small children.

The bleeding time is prolonged in systemic vascular diseases without thrombocytopenia and in vascular abnormalities with thrombocytopenia. In conditions in which the thrombocytes are less than 100 per 100 oil immersion fields on a blood smear, the bleeding time is usually prolonged. In deficiencies involving the first and second stages of coagulation the bleeding time is variable but is usually normal.

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